500 µm

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Cover photo: Living specimens of the major mesopsamic slug lineages: **A,** *Helminthope* sp. from Papua (Rhodopidae, Rhodopemorpha); **B,** *Rhodope* sp. from Belize (Rhodopidae, Rhodopemorpha); **C,** *Embletonia pulchra* (Embletoniidae, Aeolidioidea?); **D,** *Philine exigua* ('Philinidae', Cephalaspidea); **E,** *Pseudovermis salamandrops* (Pseudovermidae, Aeolidioidea?); **F,** *Philinoglossa marcusi* (Philinoglossidae, Cephalaspidea); **G,** *Platyhedyle denudata* (Platyhedylidae, Sacoglossa); **H,** *Pseudunela viatoris* (Pseudunelidae, Acochlidia); **I,** *Pontohedyle milaschewitchii* (Microhedylidae, Acochlidia) (see Jorger *et al*, p. 290–307).

Description and molecular characterization of six new species of *Nassarius* (Gastropoda, Nassariidae) from the western Pacific Ocean

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Abstract: Six new species of the genus *Nassarius* Duméril, 1805 are described, based on material collected from the Coral Triangle and the South Pacific. We combine traditional morphology-based descriptions with the molecular (Cytochrome c oxidase I - COI) signature of the new species. New species are: *Nassarius ocellatus* sp. nov. (Philippines to Vanuatu), *Nassarius houbricki* sp. nov. (Solomon Islands to Queensland and Tonga), *Nassarius radians* sp. nov. (Philippines to Vanuatu), *Nassarius vanuatuensis* sp. nov. (Vanuatu), *Nassarius velvetosus* sp. nov. (Western Australia to Fiji) and *Nassarius martinezi* sp. nov. (Solomon Islands to Tonga).

Key words: Buccinoidea, taxonomy, misspelling, species complex, protoconch

Over the last three decades, the Muséum national d'Histoire naturelle (MNHN) has carried out many expeditions to explore the benthic biota of the Indo-Pacific biogeographic region (*e.g.,* Bouchet *et al.* 2008, 2011), resulting in a fair representation of the mollusk diversity. After the molecular systematic revolution, those samplings have included DNA collections besides the traditional dried shells, thus integrating the molecular component to the international network of traditional taxonomic experts involved in the study of MNHN expedition's material (*e.g.,* Puillandre *et al.* 2010, Fedosov and Puillandre 2012, Kantor *et al.* 2012, ter Poorten 2012).

After all these years, a significant collection of Nassariidae has been brought together. The number of recent species in the family was 319 at the time of Cernohorsky (1984), the last comprehensive revision. Since then, approximately 60 new species have been described, and a few nominal species, treated as synonyms by Cernohorsky, have been revalidated. The current known diversity of the family stands at 420 valid species (see WoRMS, http://www.marinespecies.org/), of which 85% is classified in the subfamily Nassariinae; the genus *Nassarius* Duméril, 1805 alone, in a broad taxonomic extension, accounts for 336 valid species. Molecular data on Nassariidae are still meager (Couceiro *et al.* 2007, 2012, Li *et al.* 2010), and a molecular phylogeny of the Nassariinae, which will lead to a revised generic classification, is currently underway (Galindo, Ph.D. work in progress).

As with the rest of shelled mollusks, species discrimination in the Nassariidae is traditionally based exclusively on features such as shell shape and sculpture. In a minority of cases, the protoconch, radula, and egg capsules are also used as taxonomical characters (*e.g.,* Kantor and Kilburn 2001, Li *et al.* 2010, Yang and Zhang 2011). In the present paper, we describe several species regarded as new based on a suite of their shell characters, but we also include the COI sequence in the holotype designation, so they constitute the hologenophores in the sense of Pleijel *et al.* (2008).

MATERIALS AND METHODS

Abbreviations used along the text are as follow: BOLD: Barcode of Life Database; CP: Beam trawl; dd: empty shell; DW: Warén dredge; HK: Private collection H.H. Kool; juv: juveniles; lv: live-collected specimen; MNHN: Muséum national d'Histoire naturelle, Paris, France; NHMUK: British Museum of Natural History, London, UK; RMNH: Naturalis Biodiversity Center, Leiden (formerly Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands); Stn: Station number; WoRMS: World Register of Marine Species; WAM: Western Australian Museum.

Live-taken specimens were preserved in the field in 90% or 98% ethanol for molecular analysis by cutting pieces of the head-foot from anaesthetized ($MgCl_2$) specimens. In the laboratory the bodies were separated from the shells to prevent the progressive deterioration of the shells by etching. The shells were kept dry and carry the same registration number as the corresponding body or tissue in 95% ethanol.

A stereomicroscope was used to observe shell morphology and color patterns. A digital vernier (accurate to 0.1 mm) was used for measurements. Shells of the new species were photographed in dorsal, ventral and lateral view using a

Table 1. GenBank and BOLD numbers for the sequence obtained from the described new species of *Nassarius*. H: holotype, P: paratype.
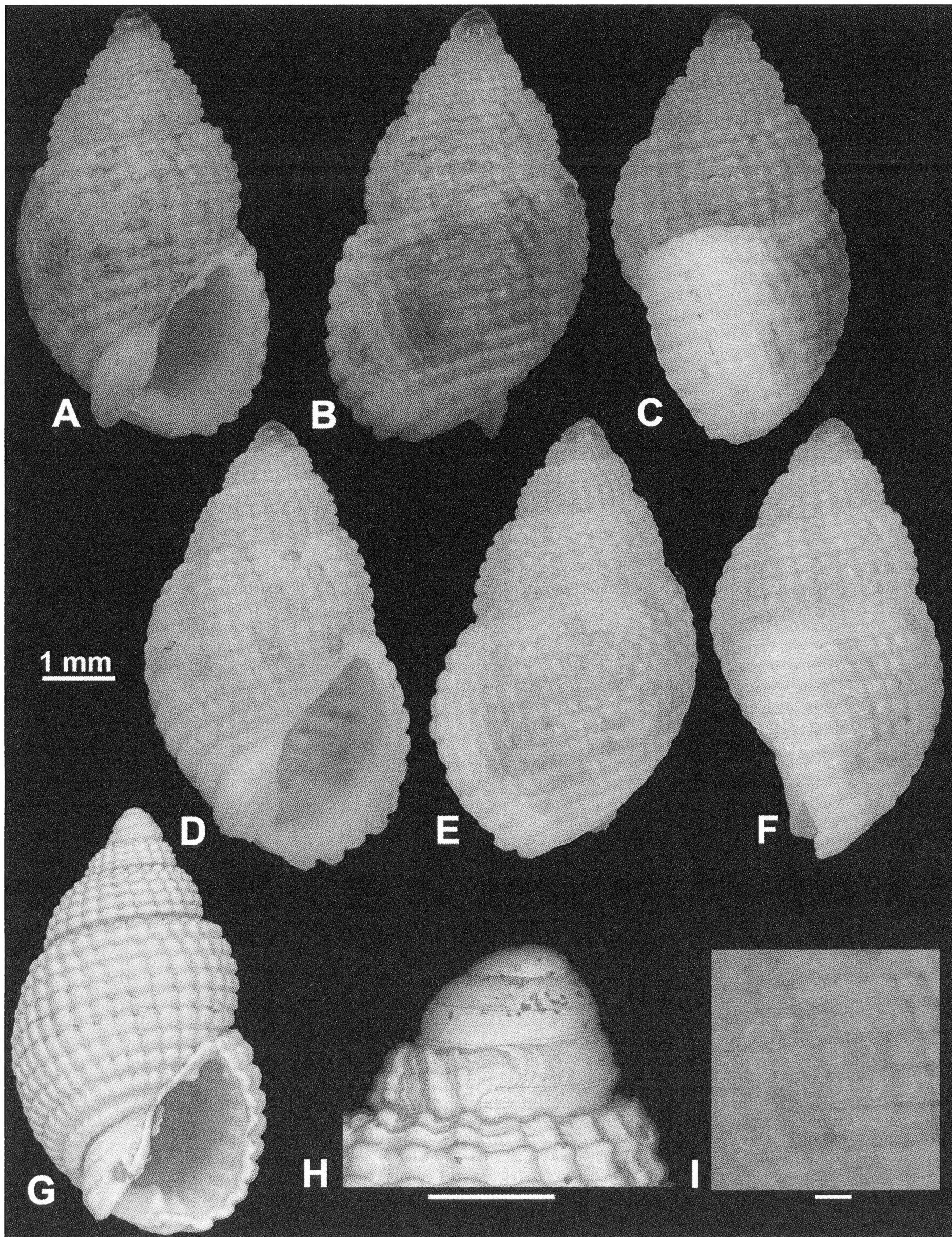
ID number	Species (sp. nov.)	Status	COI BOLD	COI GenBank
IM200731906	*Nassarius ocellatus*	H	NASSA001-12	
IM200731907	*Nassarius ocellatus*	P	NASSA002-12	
IM200736143	*Nassarius houbricki*	H	NEOGA1175-12	KC970035
IM200736147	*Nassarius houbricki*	P	NEOGA1172-12	KC970034
IM200736148	*Nassarius houbricki*	P	NASSA004-12	
IM200736179	*Nassarius houbricki*	P	NEOGA1171-12	KC970033
IM200912244	*Nassarius houbricki*		NEOGA1174-12	KC970032
IM200912277	*Nassarius houbricki*		NEOGA1186-12	KC970030
IM200912279	*Nassarius houbricki*		NEOGA1185-12	KC970031
IM200731656	*Nassarius radians*	P	NEOGA1188-12	KC970054
IM200731658	*Nassarius radians*	P	NEOGA1189-12	KC970057
IM200731659	*Nassarius radians*	P	NEOGA311-10	KC970051
IM200731660	*Nassarius radians*	P	NEOGA312-10	KC970056
IM200731672	*Nassarius radians*	P	NEOGA1173-12	KC970053
IM200731685	*Nassarius radians*	H	NEOGA334-10	KC970055
IM200731686	*Nassarius radians*	P	NEOGA335-10	KC970052
IM200731729	*Nassarius radians*	P	NEOGA360-10	KC970058
IM200731734	*Nassarius vanuatuensis*	H	NEOGA362-10	KC970062
IM200731735	*Nassarius vanuatuensis*	P	NEOGA363-10	KC970061
IM200731785	*Nassarius vanuatuensis*		NEOGA392-10	KC970060
IM200731786	*Nassarius vanuatuensis*	P	NEOGA393-10	KC970059
IM200731709	*Nassarius velvetosus*	H	NEOGA1187-12	KC970064
IM200920616	*Nassarius velvetosus*		NEOGA1178-12	KC970063
IM200734766	*Nassarius martinezi*	P	NEOGA798-10	KC970050
IM200734768	*Nassarius martinezi*	P	NEOGA799-10	KC970049
IM200735021	*Nassarius martinezi*	H	NASSA005-12	KC970038
IM200735030	*Nassarius martinezi*	P	NEOGA821-10	KC970048
IM200735031	*Nassarius martinezi*	P	NEOGA822-10	KC970047
IM200735032	*Nassarius martinezi*	P	NEOGA823-10	KC970046
IM200735965	*Nassarius martinezi*		NEOGA1177-12	KC970036
IM200735992	*Nassarius martinezi*		NEOGA857-10	KC970045
IM200735995	*Nassarius martinezi*		NEOGA1176-12	KC970037
IM200736807	*Nassarius martinezi*	P	NEOGA1183-12	KC970040
IM200912252	*Nassarius martinezi*		NEOGA1182-12	KC970041
IM200912253	*Nassarius martinezi*		NEOGA1181-12	KC970042
IM200912254	*Nassarius martinezi*	P	NEOGA1180-12	KC970043
IM200912255	*Nassarius martinezi*	P	NASSA003-12	
IM200912256	*Nassarius martinezi*	P	NEOGA1179-12	KC970044

Nikon D70S camera and, for small samples, a Nikon D5000, associated to a stereomicroscope Leica MZ16. Macro images were achieved by integrated partial focused slices by the software CombineZ5.3 (Hadley 2006). To enhance details of shell surface, a photo was taken after coating with magnesium salt. The scanning electron microscope (SEM) images of protoconchs were obtained on the Jeol JSM5410LV Low Vacuum Scanning Microscope at the Centre de Recherche sur la Conservation des Collections (MNHN-MCC-CNRS USR 3224).

Molecular sequencing was carried out at the Service de Systématique Moléculaire (MNHN- Paris VI UMR 7138 UMS 2700). Procedures from Consortium for the Barcode of Life barcoding protocols (www.barcodeoflife.org) adapted to large biodiversity collections were followed (*sensu* Puillandre *et al.* 2012). To constitute a reference tissue collection, a piece of muscle from the foot, large enough for approximately five DNA extractions, was stored in a 2D barcode tube. A second 2D tube was used to store PCR products from extraction. Extraction was done using QIAamp DNA Micro Kit (Qiagen, Stanford, CA, U.S.A.). A fragment of 658 bp of the Cytochrome Oxidase I (COI) mitochondrial gene was amplified, using the universal set of primers (LCO1490 and HCO2198) (Folmer

Figure 1. *Nassarius ocellatus,* sp. nov. **A–C,** holotype MNHN IM 2007-31906, h 5.9 mm, w 3.2 mm, Philippines, Panglao Island, 9°35′N, 123°51′E, 80 m. **D–G,** paratype MNHN IM 2009-13090, h 6.5 mm, w 3.5 mm, Philippines, Panglao Island, 9°29′N 123°52′E, 95–128 m. **H,** protoconch of paratype. **I,** Enlarged shell sculpture. Scale bars: H and I = 250 μm.

directions were sequenced to confirm accuracy of each haplotype. Sequence cleaning and alignment was done by Codon Code Aligner software. Once having validated the sequence by Blast search in the National Center for Biotechnological Information (NCBI) databases, the vouchers were submitted to the Barcode of Life Database (BOLD) and the corresponding sequences into GenBank. References to all the sequences obtained are included in Table 1.

We compiled a dataset of COI sequences available for several closely related species identified in a phylogenetic tree based on a large dataset of Nassariidae (GenBank and MNHN unpublished data) to compare with our new species' hypothesis. The analysis involved 95 COI sequences: 41 belong to the new species, 43 sequences belong to MNHN sequences from the following Nassariidae, 7 sequences were taken from GenBank depositary and 4 outgroups (Table S1; see supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0202/suppl_file/Kool_2014_Suppl.pdf).

A Bayesian analysis, consisting of two Markov chains (10 million generations each with a sampling frequency of one tree per each hundred generations) run in eight parallel analyses was performed using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). When the log-likelihood scores were found to stabilize, a consensus tree was calculated after omitting the first 25% trees as burn-in. The phylogenetic analysis was performed using the CIPRES Science Gateway (Miller *et al.* 2010). Kimura 2-parameter (K2P) distances, estimated with MEGA 5 (Tamura *et al.* 2011), were used to compare each new species with its relatives as defined above.

All the material (type series and sequences) is in MNHN unless mentioned otherwise. Detailed information on tropical

et al. 1994). PCR reactions were performed in 20 μl, containing between 1 and 2 μl of DNA, 1X reaction buffer, 2.5 mM $MgCl_2$, 0.26 mM dNTP, 0.3 μM of each primer, 5% DMSO and 5% BSA (10 mg/l) and 1.5 units of Q-Bio Taq, (QBiogene, Carlsbad, CA, U.S.A.). Thermocycles used for COI gene are those described in Hebert *et al.* (2003). Annealing temperature was 54 °C for 40 seconds. Sequencing was carried out by the Centre National de Séquençage (Genoscope-France). In all cases, both

deep sea surveys program run by MNHN (from which we obtained the new species described) can be found in Bouchet *et al.* (2008).

SYSTEMATICS

Family Nassariidae Iredale, 1916 (1835)
Subfamily Nassariinae Iredale, 1916 (1835)
Genus *Nassarius* Duméril, 1805
Type species (by subsequent monotypy, Froriep, 1806). — *Buccinum arcularia* Linnaeus, 1758. Recent.

Nassarius ocellatus new species
(Figs. 1 and 2)

Type material

Holotype MNHN IM 2007-31906; BOLD ID = NASSA001-12 (Fig. 1A–C) (height 5.9 mm, width 3.2 mm). Eight paratypes MNHN: Philippines, West Pamilacan Island, 9°29′N, 123°52′E, 95–128 m, 1 dd, height 6.5 mm, width 3.5 mm (MNHN IM 2009-13090) (Fig. 1D–H); 1 dd (MNHN IM 2000-25418); Philippines, Panglao Island, 9°35′N, 123°51′E, 80 m, 1 lv (MNHN IM 2007-31907); Philippines, Bohol Sea, off Balicasag Island, 9°32′N, 123°42′E, 111–115 m, 1 dd (MNHN IM 2000-25420); Philippines, 16°04′N, 121°56′E, 111–113 m, 4 dd (MNHN IM 2000-25419).

Figure 2. Geographical and bathymetrical distribution of *Nassarius ocellatus* sp. nov. Each bar represents all lv or dd specimens. Star indicates type locality.

Type locality

Philippines, Panglao Island, Biking, Catarman, 9°35′N 123°51′E, 80 m.

Additional material (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0202/suppl_file/Kool_2014_Suppl.pdf)

Description

Shell oval and glossy. Height 5.9 mm and width 3.2 mm. Planktotrophic protoconch with 3 whorls, shiny and carinated (Fig. 1H protoconch of paratype MNHN IM 2009-13090). First 1.5 whorls colored in intense reddish brown. Teleoconch of 3.5 convex whorls, last whorl bulbous. Whole shell longitudinally ribbed. Last whorl with approximately 25 axial ribs, equally strong and evenly spaced; spiral sculpture consists of 6 deep spiral grooves on the penultimate whorl and 10 on the last whorl. Ribs and grooves result in quadrangular tubercles and glassy interspaces (Fig. 1I). Siphonal area with 3 cords. Siphonal canal short. Suture deeply impressed and channeled. Aperture oval, outer lip slightly thickened, not variced, crenulated, 10 lirae within. Columella slightly calloused, smooth, parietal denticle strong, anal canal prominent. Color whitish to creamy yellowish, reddish brown mottled; last axial ribs of body whorl, aperture and columella white.

Intraspecific variation: All available adult specimens are comparable in size and color.

Distribution

Nassarius ocellatus sp. nov. was found alive in 80 m, empty shells are recorded down to 201 m. The species is known from the Philippines, Indonesia, Vanuatu and the Solomon Islands (Fig 2).

Discussion

The combination of the shape, the tubercular sculpture of quadrangular knobs, the reddish part of the protoconch and the crenulated outer lip make *Nassarius ocellatus* sp. nov. easy to distinguish from all other known species in the family Nassariidae. *Nassarius ocellatus* sp. nov. is figured in Poppe (2008: 125, plate 357, Fig. 2) as *N. multigranosus* (Dunker, 1847), but according to Tsuchiya (*in* Okutani 2000: 449; plate 223, Fig. 61) that species is granulated and larger sized. The lectotype of *N. multigranosus* is figured by Cernohorsky (1984: plate 39, Fig. 1). The axial ribs are stronger, the granules are smaller and the protoconch is sharper. The distribution of *N. multigranosus* is limited to Japan and Korea.

Etymology

From the Latin word *ocellus* = little eye, with reference to the chestnut brown "eye" at the top of the protoconch.
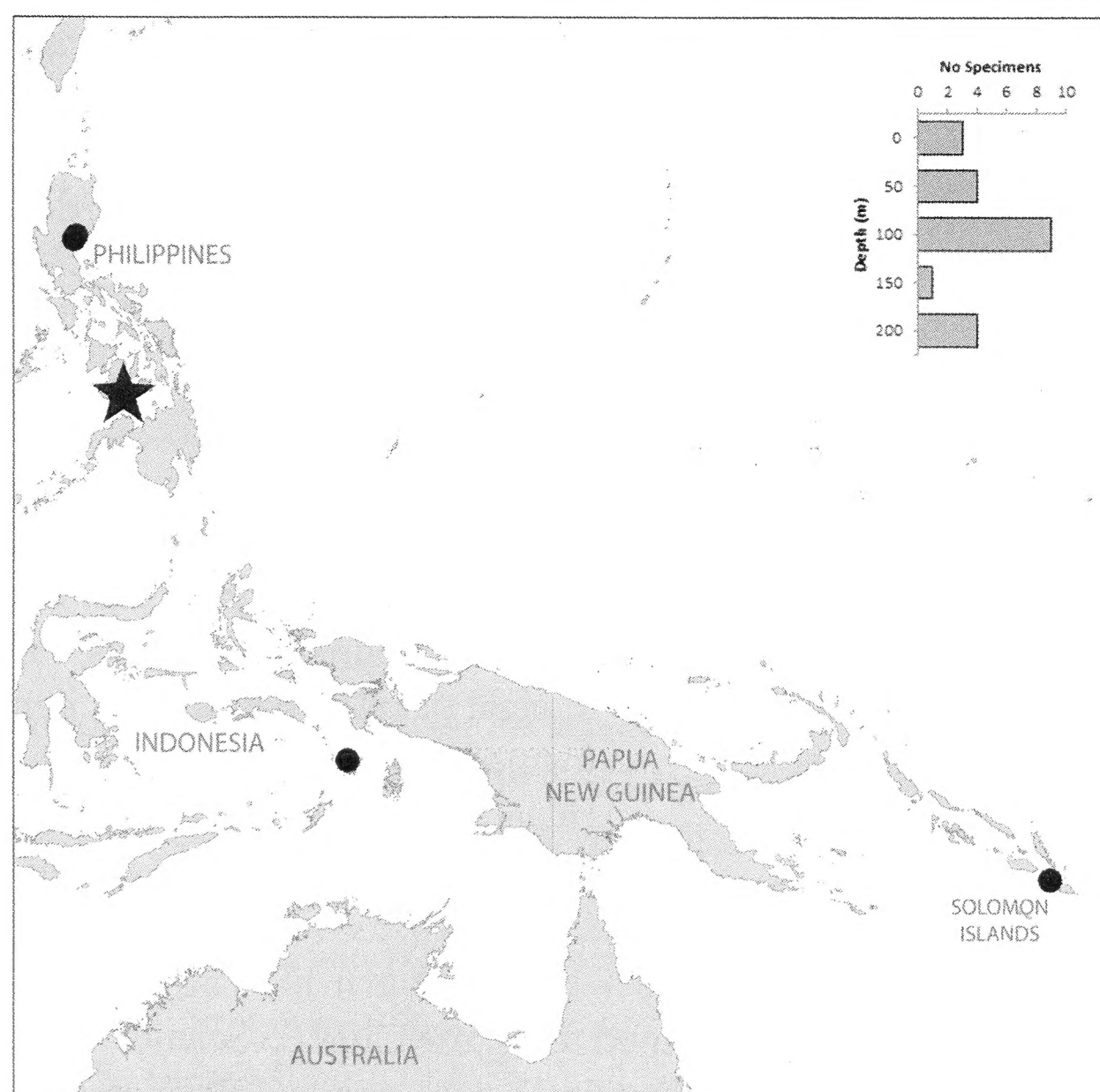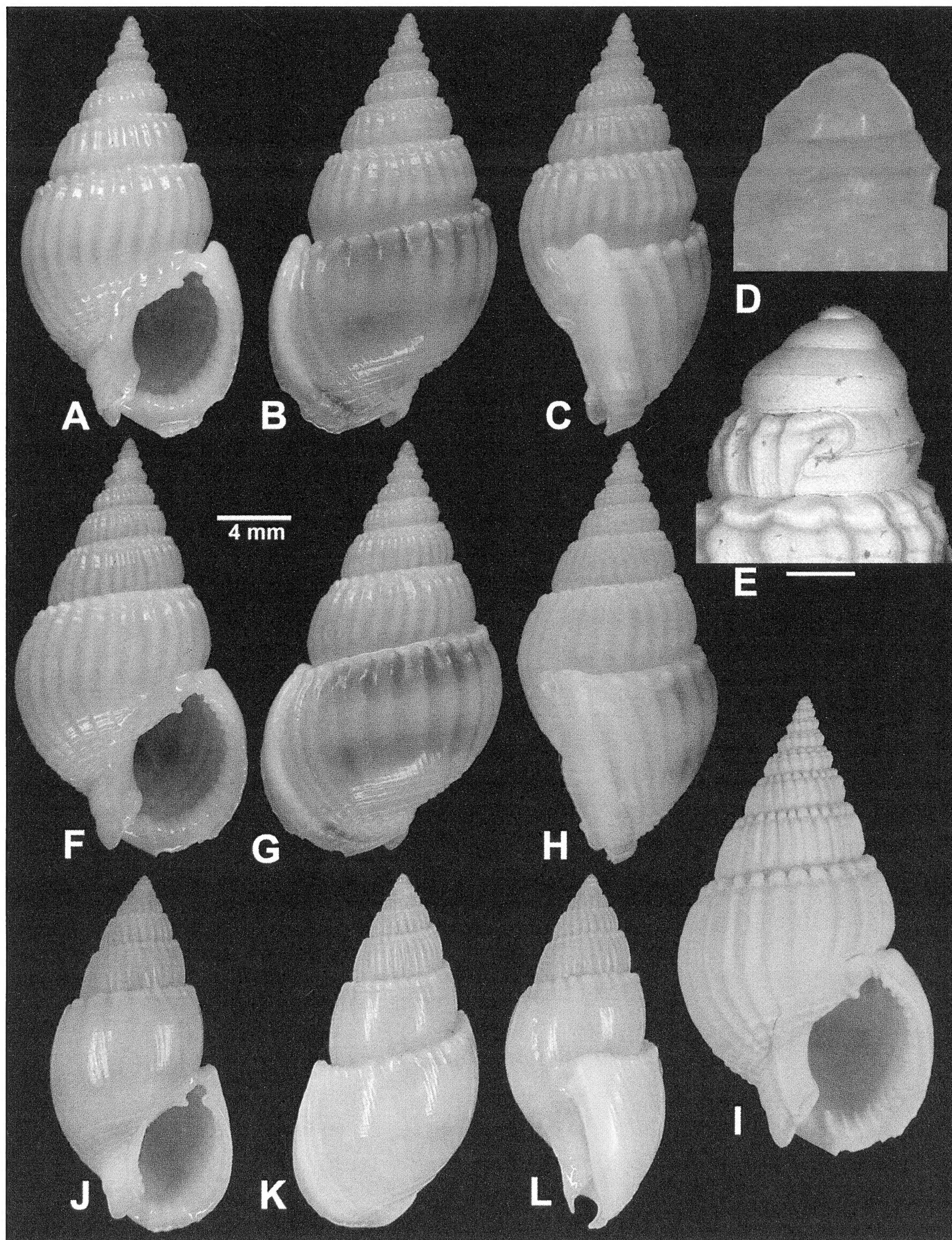
Figure 3. *Nassarius houbricki,* sp. nov. **A–E,** holotype MNHN IM 2007-36143, h 22.6 mm, w 11.4 mm, Solomon Islands, East Malaita, SALOMONBOA 3, stn CP2804, 09°15′S, 161°21′E, 150–175 m. **D–E,** protoconch of the holotype, scale bar: D 250 μm. **F–I,** paratype MNHN IM 2007-36148, h 28.3 mm, w 13.1 mm, Solomon Islands, East Malaita, SALOMONBOA 3, stn CP2804, 09°15′S, 161°21′E, 150–175 m. *Nassarius alabasteroides* Kool 2009. **J–L,** holotype MNHN IM 2000-21889, h 24.7 mm, w 11.6 mm, Coral Sea, Banc Capel, MUSORSTOM 5: stn DW270, 24°49′S, 159°34′E, 223 m.

Nassarius houbricki new species
(Figs. 3 and 4)

Type material

Holotype MNHN IM 2007-36143; BOLD ID = NEOGA 1175-12; GenBank accession number for COI = KC970035

(Fig. 3A–E) (height 22.6 mm, width 11.4 mm). Four paratypes MNHN: Solomon Islands, East Malaita, SALOMONBOA 3, stn CP2804, 09°15′S, 161°21′E, 150–175 m, 1 lv, height 28.3 mm, width 13.1 mm (MNHN IM 2007-36148) (Fig. 3F–I); 1 lv juv (MNHN IM 2007-36147); 1 lv juv (MNHN IM 2007-36179); Solomon Islands, South Malaita, SALOMONBOA 3, stn CP2814, 09°45.00′S, 161°33′E, 375–431m, 1 dd juv (MNHN IM 2000-25425).

Type locality

Solomon Islands, East Malaita, 09°15′S, 161°21′E, 150–175 m [SALOMONBOA 3, stn CP2804].

Additional material (See supplemental document at: http://www. bioone.org/doi/suppl/10.4003/ 006.032.0202/suppl_file/Kool_ 2014_Suppl.pdf)

Description

Shell elongate ovate, height 22.6 mm, width 11.4 mm, thin and shining. Planktotrophic protoconch (Fig. 3D–E) approximately 3.3 whorls, last 1.5 carinated. Teleoconch 7.5 convex whorls, regularly ribbed, dorsal side of last whorl with ribs only unto periphery, lower part smooth, aside from 3 axial ribs towards outer lip, last one prominent; edge of outer lip thin. Suture channeled, at the last whorl accentuated by ribs higher than bottom of channel. Penultimate whorl with approximately 30, last whorl with 22 ribs. First 5 teleoconch whorls with 1 to 2 prominent spiral cords, strongest one below suture, causing fine glistening beads near suture; last 2 whorls with shallow groove below suture, getting less prominent towards outer lip. Five spiral grooves at base, siphonal area with 3 cords.

Aperture oval, outer lip thin, inside with approximately 15 short, evenly-sized lirae. Columella with 1 to 2 weak folds,
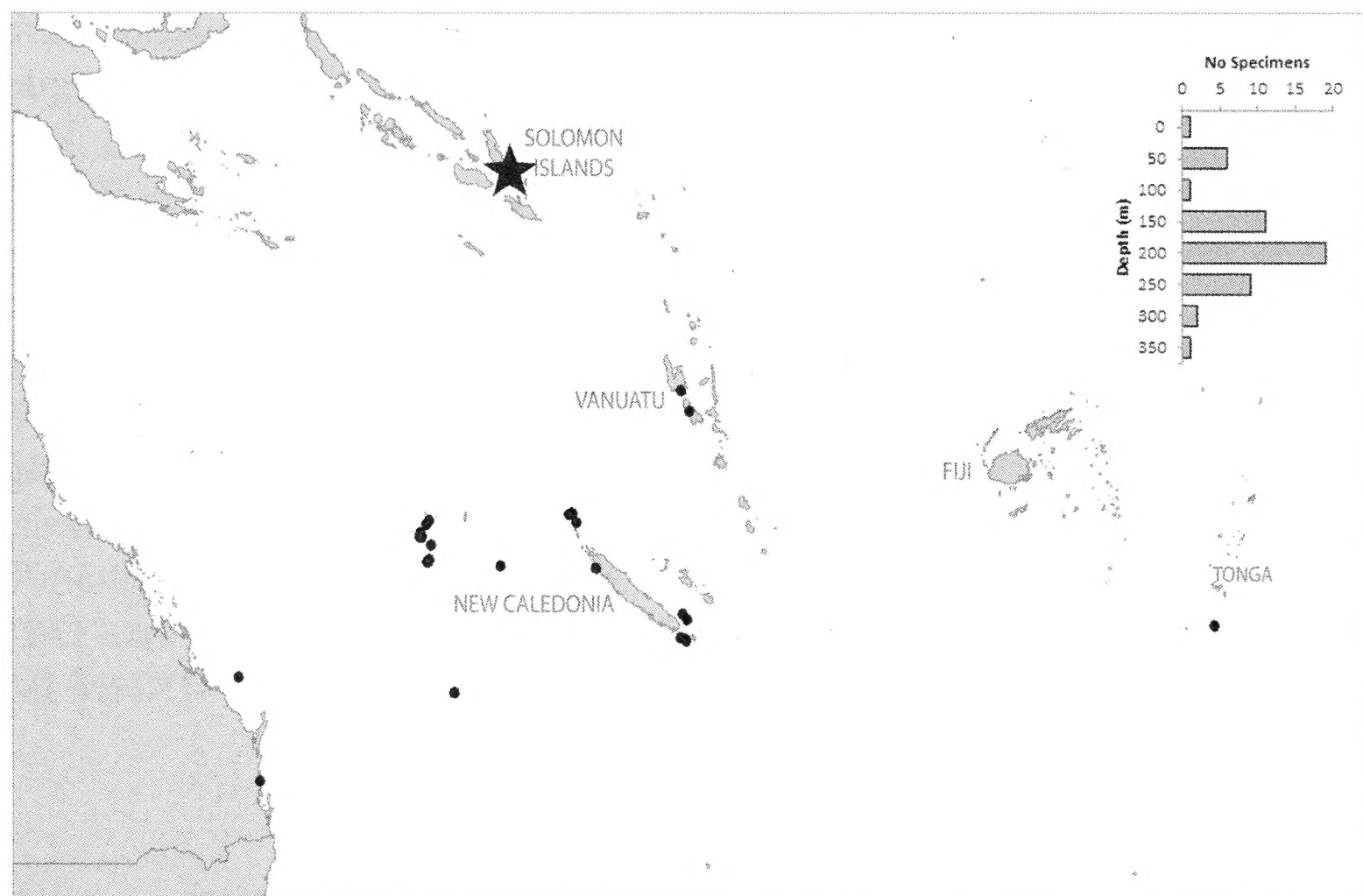
Figure 4. Geographical and bathymetrical distribution of *Nassarius houbricki* sp. nov. Each bar represents all lv or dd specimens. Star indicates type locality.

basal cords visible; callus anteriorly laminate, posteriorly thinning and slightly extending over last whorl. Some callosity near anal canal; parietal denticle prominent, anal canal wide. Operculum not present in holotype, yellow in other specimens.

Intraspecific variation: Color varies from white to pale yellow, on dorsal side of last whorl, below suture, a chestnut-brown band; on periphery and at base a paler band. Size of adult specimens from 22.5 mm to 30.5 mm.

Distribution

Found in Australia (Queensland), New Caledonia, Vanuatu, Solomon Islands and Tonga (Fig. 4). Coral sand at depths, ranging from 62 to 375 m.

Discussion

Nassarius houbricki sp. nov. may be compared with *Nassarius siquijorensis* (A. Adams, 1852) and *Nassarius alabasteroides* Kool, 2009. Cernohorsky (1991: 201) misidentified specimens of *Nassarius alabasteroides* Kool, 2009 (Fig. 3J–L) and *N. houbricki* sp. nov. as *Nassarius (Zeuxis) siquijorensis* (A. Adams, 1852). *Nassarius siquijorensis* (discussed and figured by Kool (2007: 89; Figs 17–20)) is finely ribbed all over the shell and not as thin and light in weight as *N. houbricki* sp. nov. The distribution of *N. siquijorensis* as established by Cernohorsky (1991: 202) ranges from the Red Sea to Japan and New Caledonia, but Kool (2009: 98) showed that it is limited to the western Pacific; it occurs neither in Japan nor in the Red Sea. Tsuchiya (*in* Okutani 2000: pl. 221, Fig. 35) figured a specimen of *N. euglyptus* (Sowerby III, 1914) from Japan under the name *N. siquijorensis* (Kool

2007: 89; Figs. 13–16). Also, the occurrence of *N. siquijorensis* in New Caledonia is a misidentification with *N. alabasteroides* Kool 2009 [Lots mentioned by Cernohorsky (1991: 201–202): DC63, DC84, DC66, DC67, 258, 263, 265, 266, 270, 276, 289, 293, 294, 295, 296, 312, 315, 318, 320 and CC175] and *N. houbricki* sp. nov. [Lots mentioned by Cernohorsky (1991: 201–202): DC 33, CP10, 284, 347, DW151, DW186 and DW367]. *Nassarius alabasteroides* is distinguished from *N. houbricki* sp. nov., because is more elongate, taller, lighter and by its less prominent ribs.

Loch (1992: 1) figured *Nassarius houbricki* sp. nov. under the name *Nassarius* cf. *psila* (Watson, 1882), from Queensland, off Raine Islands at a depth of 284 m. Cernohorsky (1984: pl. 2, Figs 9–10) and Kaicher (card 3467) figured the holotype of *N. psila* and there are several differences between the two species: *N. psila* is smaller, has finer axial ribs on the early whorls and has no ribs on the ventral side of the last whorl. *Nassarius houbricki* sp. nov. has strong ribs on the early whorls as well as on the ventral side of the last whorl; it is also light in weight, but not 'very thin' as Kaicher (card 3467) describes *N. psila*.

Etymology

The species named in honor of the late Richard "Joe" Houbrick (1937–1993), Curator at the Mollusk Department at the National Museum of Natural History, Smithsonian Institution, in recognition for his extensive contribution to malacology (Harasewych and Kabat 1995). He was also the Ph.D. thesis advisor of the senior author's son Silvard Kool.

Nassarius radians new species
(Figs. 5 and 6)

Type material

Holotype MNHN IM 2007-31685; BOLD ID = NEOGA 1188-12; GenBank accession number for COI = KC970054 (Fig. 5A–C, H) (height 19.3 mm, width 9.8 mm). Ten paratypes MNHN, 2 paratypes HK171.01: Vanuatu, Canal du Segond, SANTO 2006, stn AT82, 15°31.6′S, 167°12.4 ′E, 58–59 m, 1 dd, height 20.0 mm, width 10.8 mm (MNHN IM 2009-13091) (Fig. 5D–G); Vanuatu, North Urelapa Island, SANTO 2006, stn AT29, 15°35.9′–36.0′S, 167°01.3′–01.6′E, 83–90 m, 1 lv (MNHN IM 2007-31729); 1 lv (MNHN IM 2007-31686); Vanuatu, East Urelapa Island, SANTO 2006, stn AT41, 15°36.7′–37.0′S, 167°02.7′–02.8′E, 88–118 m, 3 dd (1 MNHN IM 2000-25430, 2 HK 171.01); Vanuatu,
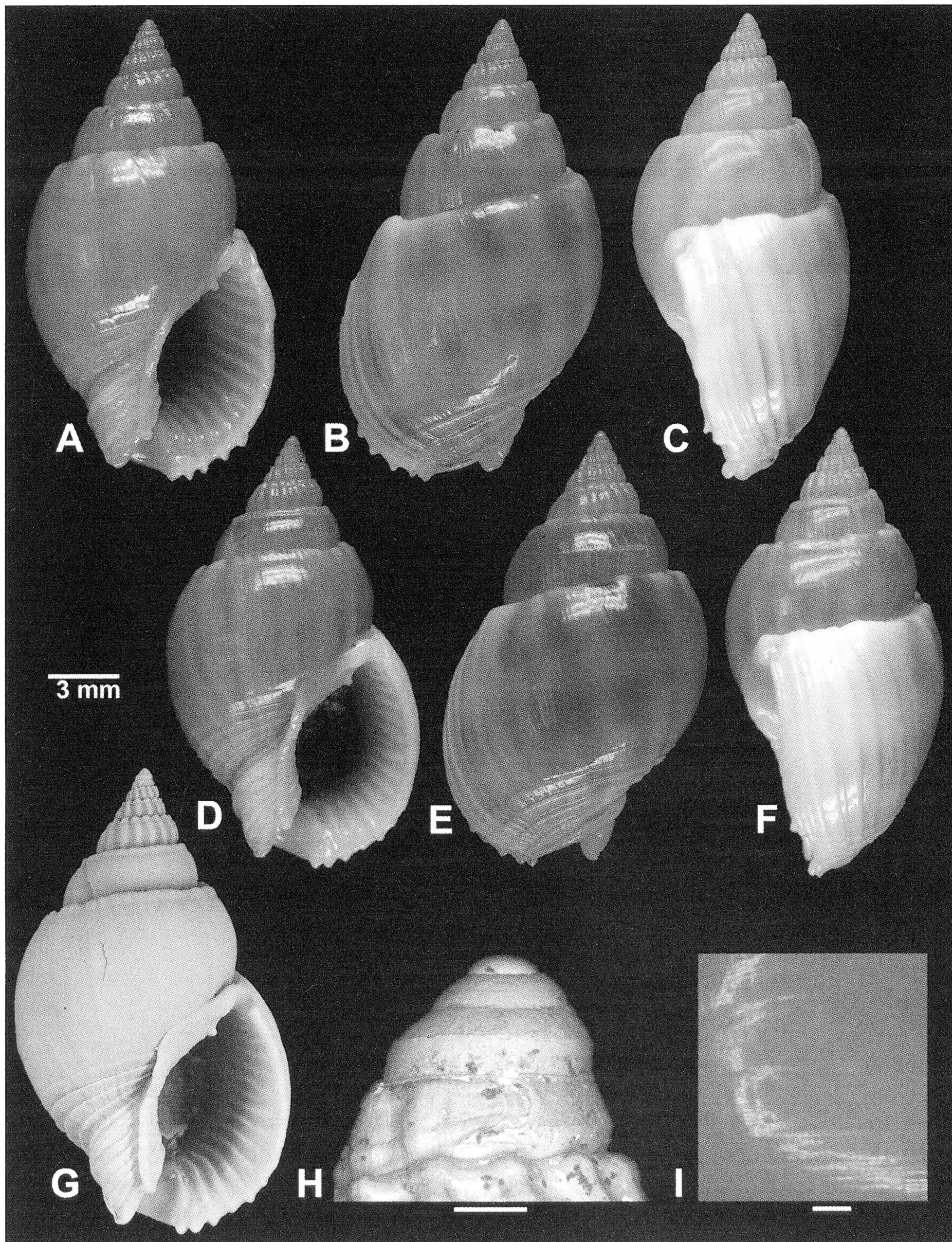
Figure 5. *Nassarius radians*, sp. nov. **A–C**, holotype MNHN IM 2007-31685, h 19.3 mm, w 9.8 mm, Vanuatu, Segond Channel, SANTO 2006, stn AT82, 15°32′S, 167°12′E, 58–59 m. **D–G**, paratype MNHN IM 2009-13091, h 20.0 mm, w 10.8 mm, Vanuatu, Segond Channel, SANTO 2006: stn AT82, 15°32′S, 167°12′E, 58–59 m. **H**, protoconch of the holotype. **I**, Enlarged shell sculpture. Scale bar: H and I = 250 μm.

Type locality

Vanuatu, Canal du Segond, 15°31.6′S, 167°12.4′E, 58–59 m [SANTO 2006, stn AT82].

Additional material (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0202/suppl_file/Kool_2014_Suppl.pdf)

Description

Shell elongate ovate, extremely shining, height 19.3 mm, width 9.8 mm. Planktotrophic protoconch of 3.75 smooth whorls, last 1.5 carinated (Fig. 5H). Teleoconch of 5.5 whorls, first 3 axially ribbed, other ribs smooth. Suture deeply channeled. First three whorls with a subsutural spiral groove, axial ribs coronated. On next whorls subsutural spiral groove gradually indistinct, suture with undefined crenulations. Base of last whorl with 5 axially striated spiral grooves. Towards edge of outer lip 3–5 low axial ribs, lip not thickened, with 4–5 prominent denticles. Inside lip with 14 strong and deep lirae, siphonal area with 5 prominent, axially striated spiral grooves.

Columella laminate and with an abapical tooth, inside with 15 lirae. Parietal denticle strong, anal canal wide and prominent.

Color bright light brown (Fig. 5I) with darker axial streaks, more prominent at the suture, and with 2 lighter spiral bands on the last whorl. Outer lip and columella white, inside aperture brown.

Intraspecific variation: Dead collected specimens have lost their brilliant shine and are off white. Size varies from about 18.3 mm to 27.5 mm.

Distribution

Philippines, the Solomon Islands and Vanuatu (Fig. 6), alive at depths between 53–180 m.

Canal du Segond, SANTO 2006, stn AT53, 15°31.8′S, 167°13.6′E, 62–71 m, 1 lv (MNHN IM 2007-31656); 1 lv (MNHN IM 2007-31658); Vanuatu, Northeast Urelapa Island, SANTO 2006, stn AT119, 15°36′S, 167°02′E, 87–120 m, 1 lv (MNHN IM 2007-31659); 1 lv (MNHN IM 2007-31660); 1 lv (MNHN IM 2007-31672); 1 dd (MNHN IM 2000-25436).
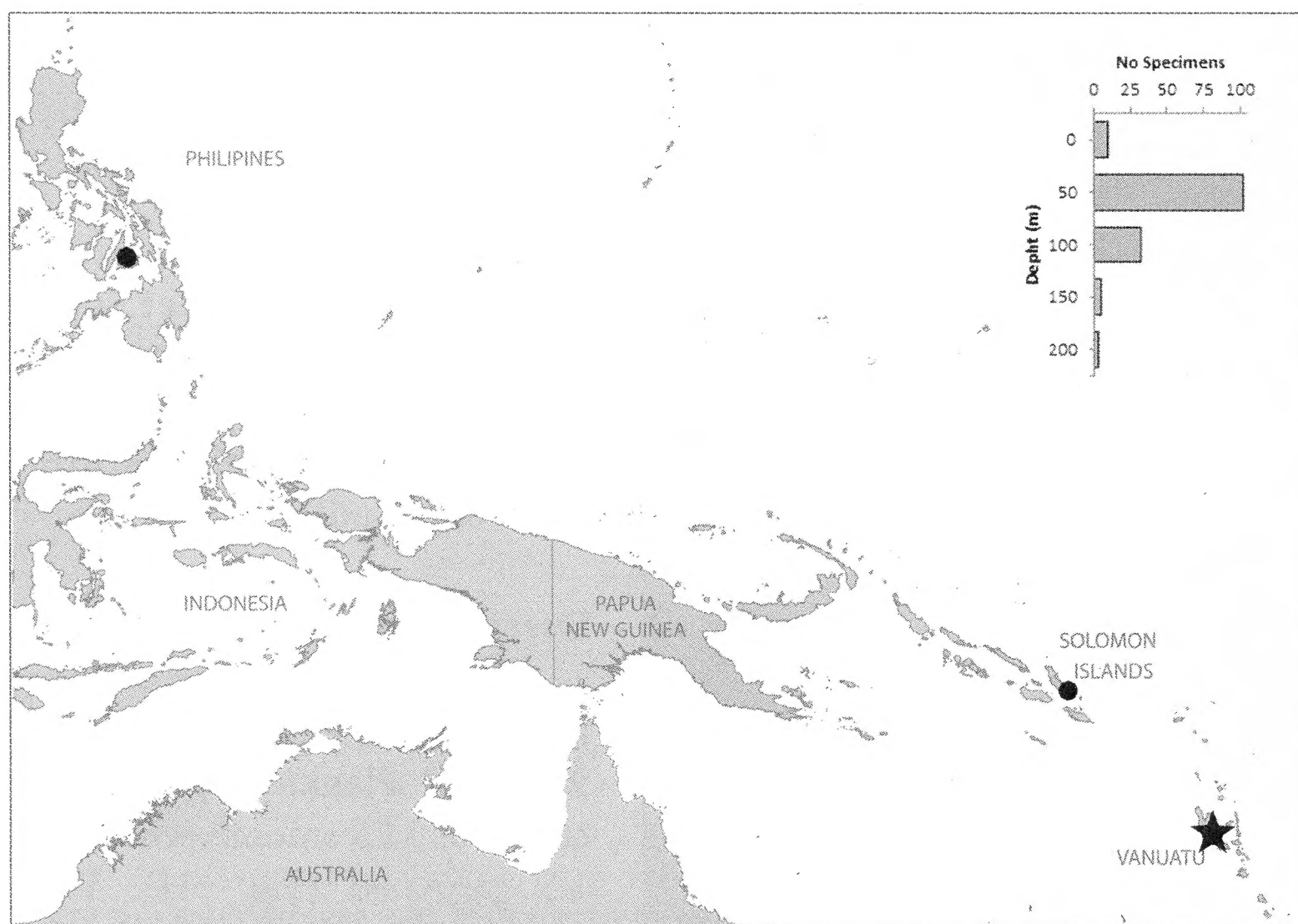
Figure 6. Geographical and bathymetrical distribution of *Nassarius radians* sp. nov. Each bar represents all lv or dd specimens. Star indicates type locality.

Discussion

Live-taken specimens of *N. radians* sp. nov. are easy to identify by their brilliant shine, the characteristic color pattern and the last 2 whorls of the shell which are nearly smooth. A species comparable with *N. radians* sp. nov. is *N. thachi* Dekker, 2004, which can be distinguished from *N. radians* sp. nov. by its more elongate shape and its size: up to 36 mm. Moreover, *N. thachi* has a clear subsutural groove. The recently described *Nassarius* (*Zeuxis*) *nanhaiensis* Zhang, 2013, from the South China Sea, at first sight, looks, very much like *N. radians* sp. nov. The latter differs however, in the number of protoconch whorls: 3.75 against 2–2.50 in *N. nanhaiensis*. The protoconch of *N. nanhaiensis* is described as smooth, whereas *N. radians* sp. nov. is prominently carinated. Other differences are the number of the axial ribs on the first 2 to 3 teleoconch whorls and the color of the shell. *Nassarius radians* sp. nov. is extremely glistening.

Another species with similarities with *N. radians* sp. nov. is *Nassarius comptus* (A. Adams, 1852) (Fig. 7A–D). *Nassarius radians* sp. nov. can be distinguished from *N. comptus* by the conspicuously different ribs on the three penultimate whorls: *N. comptus* has thick ribs, whereas the ribs of the new species are less prominent. The protoconch has fewer whorls, the callus is strictly limited to the columella and the shell is less shiny than in *N. radians* sp. nov.

The redescription of *N. comptus* by Cernohorsky (1984) is based on a taxonomic concept that includes several nominal species as synonyms, making comparison with *N. radians* sp. nov. somewhat problematic. The lectotype NHMUK 1969531/1 (Fig. 7A–D) designated by Cernohorsky (1984: 146), and the syntype NHMUK 1969531/2 (Fig. 7E–H) of *N. comptus*, are

illustrated here for the first time in color. A comparison of the lectotype of *N. comptus* with the holotype NHMUK 1973021 of *Nassa cinnamomea* (A. Adams, 1852) (Fig. 12G–H) shows that the latter is distinguished from *N. comptus* by its slenderer shape and the presence of a subsutural groove. They represent two different species in our opinion. The first three teleoconch whorls of the lectotype of *N. comptus* (Fig. 7C–D) are far more prominently ribbed than those of the syntype (Fig. 7G–H). Also the taller shape, the callus and the bands mismatch. In this respect, the paralectotype of *N. comptus* seems to be a specimen of *N. haldemani* (Dunker, 1847), which species is also illustrated in Fig. 12A–C.

The taxonomic status of *N. comptus* and the great number of taxa considered synonyms by Cernohorsky (1984: 146), correctly or incorrectly, is such a complex problem that it exceeds the objectives of the present paper and deserves a separate study for the future. Kool (2006: 98), supported on the discussion of Kase and Kinjo (1996: 199), already noticed the erroneous synonymisation of *Nassarius cinnamomea* with *N. comptus*.

Etymology

Nassarius radians sp. nov., from the Latin *radiare* = shine, named after its brilliant shine.

Nassarius vanuatuensis new species
(Figs. 8 and 9)

Type material

Holotype MNHN IM 2007-31734; BOLD ID = NEOGA 362-10; GenBank accession number for COI = KC970062 (Fig. 8A–C, H) (height 6.7 mm, width 3.1 mm). Eleven paratypes MNHN, 3 paratype HK 173.02: Vanuatu, West Tangao Island, SANTO 2006, stn LD28, 15°35.4′S, 166°58.7′E, 3–8m, 1 dd, height 6.4 mm, width 2.9 mm (MNHN IM 2009-13089) (Fig. 8D–G); 1 lv (MNHN IM 2007-31735); 1 lv (MNHN IM 2007-31786); 1 dd (MNHN IM 2000-25446); Vanuatu, Canal du Segond, SANTO 2006, stn EP03, 15°32.2′S, 167°09.06′E, 46 m, 1 dd (MNHN IM 2000-25441); Vanuatu, Canal du Segond, SANTO 2006, stn ED02, 15°31.07′S, 167°09.7′E, 18–21 m, 2 dd (1 MNHN IM 2000-25442, 1 HK 173.02); Vanuatu, Canal du Segond, SANTO 2006, stn ED17, 15°32′S, 167°10′E, 23–27 m, 2 dd (MNHN IM 2000-25443); Vanuatu, Tangoa Island, SANTO 2006, stn FB68, 15°35.4′S, 166°59.7′E, 11 m, 1 dd (MNHN IM-2000-25444); Vanuatu, Tangoa Island, SANTO 2006, stn LD27, 15°35.3′S, 166°59.3′E, 3–5 m, 3 dd (1 MNHN IM 2000-25445, 2 HK 173.01); Vanuatu, Tangoa Island, SANTO 2006, stn LD39, 15°35.4′S, 166°58.7′E, 6–9 m, 1 dd (MNHN IM 2000-25447).
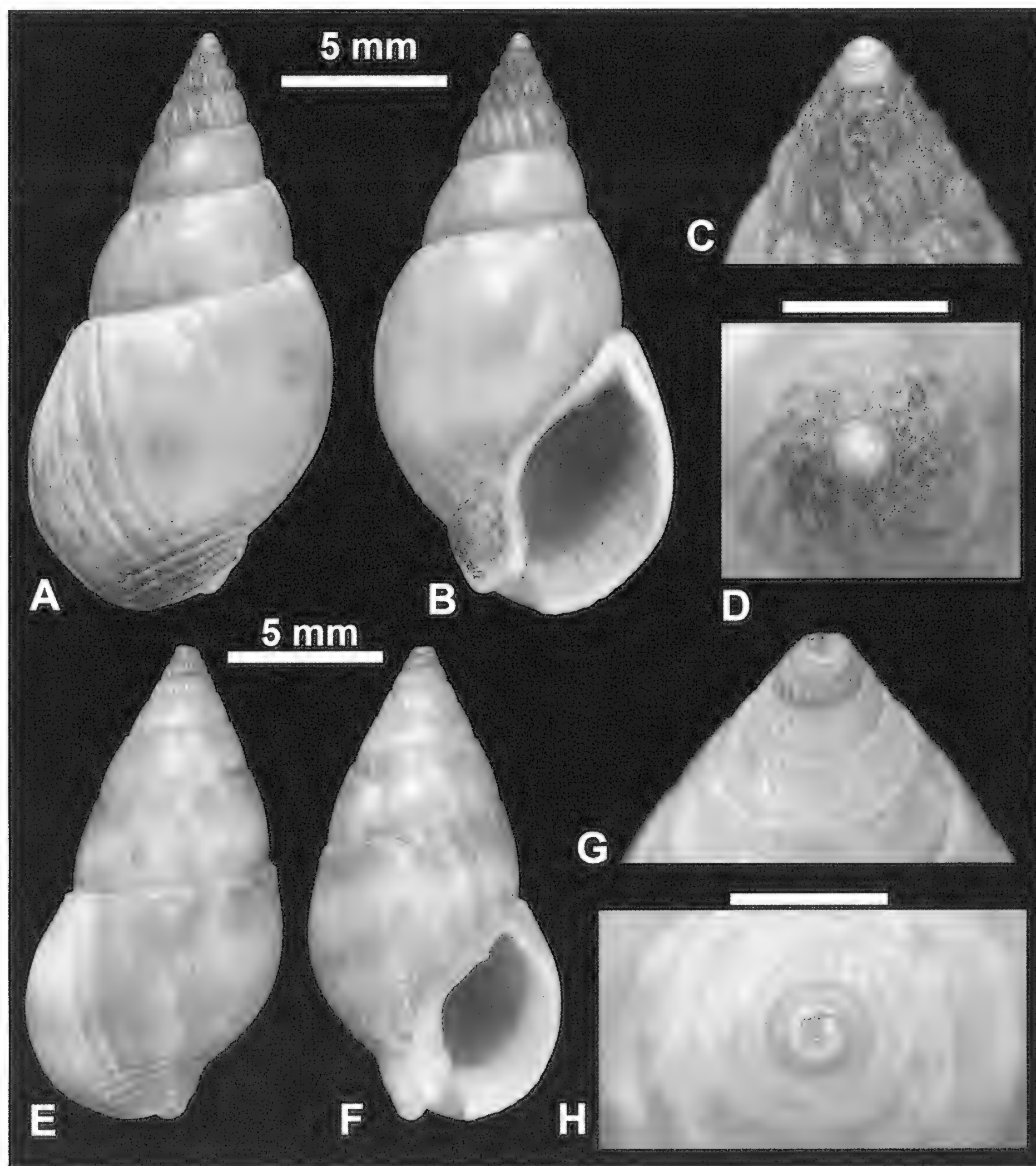
Figure 7. *Nassa compta* A Adams, 1852. **A–D**, lectotype NHMUK 19695311, h 18.8 mm, Cape St. Antonio, South Africa (= ? error). **E–H**, syntype NHMUK 19695312. Scale bar: C, D, G and H = 2 mm.

consisting of a deep subsutural groove, forming strong nodules on ribs at the suture; 3 to 4 overriding spirals on the first 3 teleoconch whorls; on last whorl, spirals are no more overriding the ribs, but only present in interspaces (Fig. 8I); 5 overriding basal cords, siphonal area with 4 to 5 cords.

Aperture oval, outer lip with about 10 lirae inside. Columella plicate throughout. Columellar callus well bordered, parietal denticle strong, anal channel deep and narrow.

Shell light brown, with 3 darker spiral bands, varix white.

Intraspecific variation: Color light brown to dark brown. Color bands can be absent or indistinct. Length varies from 6.0 mm to 8.3 mm.

Distribution

Nassarius vanuatuensis sp. nov. is only known from Vanuatu (Fig. 9), alive in 3–8 m. Empty shells are found regularly to 46 m; one shell in 127–193 m was most probably carried downslope.

Discussion

Nassarius vanuatuensis sp. nov. can be distinguished from species belonging to the complex of *Nassarius pauper* (Gould, 1850) by its carinate protoconch and by the slightly convex and more slender shape of the shell. Additionally, species of the *pauper* group have secondary spirals between the more prominent primary ones.

Another comparable species also occurring in Vanuatu is *Nassarius moestus* (Hinds, 1844). This species was illustrated by Cernohorsky (1984: lectotype plate 42, Fig. 10) and Poppe (2008, color plate 357, Fig. 3). *Nassarius moestus* has lower axial ribs with over-riding spirals and a shiny, reddish-brown and not well-bordered callus, which extends partly over the body whorl. In addition, the shell of this species is similar in size to that of *N. vanuatuensis* sp. nov, but more convex.

Type locality

Vanuatu, West Tangoa Island, 15°35.4′S, 166°58.7′E, 3–8m [SANTO 2006, stn LD28].

Additional material

(See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0202/suppl_file/Kool_2014_Suppl.pdf)

Description

Shell elongate, slender, height 6.7 mm, width 3.1 mm. Planktotrophic protoconch with 3 carinated, milky whorls (Fig. 8H). Teleoconch with 4.3, slightly convex whorls; penultimate whorl with 17 ribs, last whorl with 15 prominent ribs; varix strong. Suture moderately ledged; spiral sculpture

Etymology

The species is named after the archipelago of Vanuatu, where the type locality is.
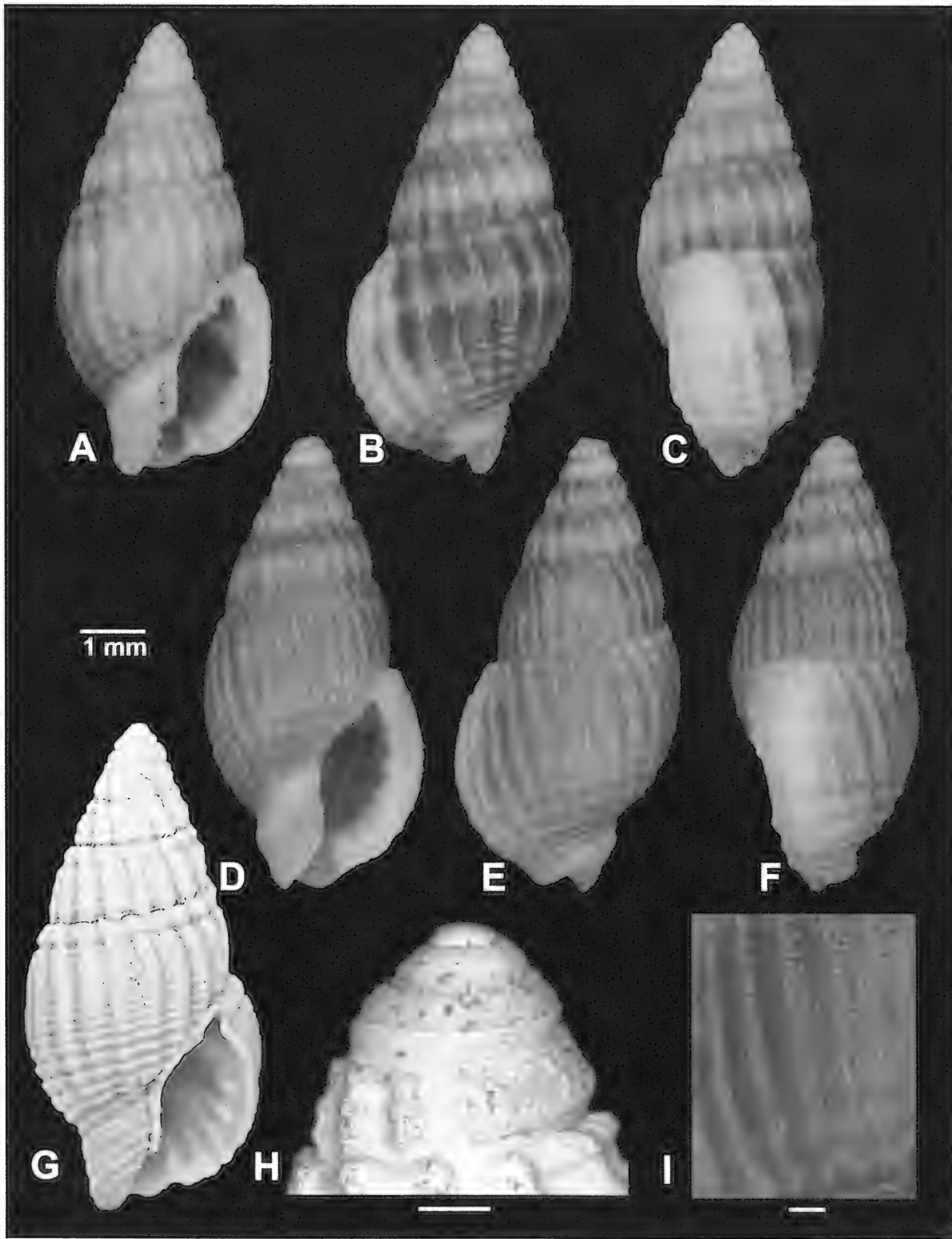
Figure 8. *Nassarius vanuatuensis*, sp. nov. **A–C**, holotype MNHN IM 2007-31734, h 6.7 mm, w 3.1 mm, Vanuatu, West Tangao Island, SANTO 2006: stn LD28, 15°35'S, 166°59'E, 3–8 m. **D–G**, paratype MNHN IM 2009-13089, h 6.4 mm, w 2.9 mm, Vanuatu, West Tangao Island, SANTO 2006: stn LD28, 15°35'S, 166°59'E, 3–8 m. **H** protoconch of the holotype. **I**, Enlarged shell sculpture. Scale bar: H and I = 250 μm.

MNHN, 1 paratype HK 159.03: Vanuatu, South Urelapa Island, SANTO 2006, stn EN20, 15°37.2'S, 167°01.9'E, 85 m, 1 dd, height 22.8 mm, width 10.3 mm (MNHN IM 2009-13088) (Fig. 10D–H); 3 dd (MNHN IM 2000-25550); Vanuatu, Westnorthwest Urelapa Island, SANTO 2006, stn AT46, 15°38'S, 167°05'E, 92–104 m, 1 dd (MNHN IM 2000 25551); Vanuatu, Malo Island, SANTO 2006, stn LD20, 15°42.7'S, 167°15.1'E, 2–5 m, 1 dd (MNHN IM 2000-25552); Vanuatu, Southeast corner of Santo, SANTO 2006, deep water dredging, 2 dd (1 MNHN IM 2000-25553, 1 HK 159.03).

Type locality

Vanuatu, South Urélapa Island, 15°37'S, 167°02'E, 116 m [SANTO 2006, stn EN25].

Additional material (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0202/suppl_file/Kool_2014_Suppl.pdf)

Description

Shell slender, elongate-ovate, velvety shining, height 22.0 mm, width 10.1 mm. Planktotrophic protoconch with 3.5 transparent whorls; the last keeled (Fig. 10H from paratype MNHN IM 2009-13088). Holotype's protoconch is incomplete. Teleoconch with 5.5 slightly convex whorls, first 3 finely ribbed and showing 4 to 6 fine spiral grooves. The rest of the whorls smooth, except a fine spiral groove below the suture and microscopic growth lines. Suture narrowly ledged.

Last whorl with 7 to 8 spiral grooves at the base and approximately 7 low ribs near outer lip. Outer lip with 4 to 5 denticles and approximately 15 evenly sized lirae inside, not variced and with a narrow edge of callus. Aperture oval. Columellar callus thick, marginated and narrow, following the aperture from the siphon to the anal canal, anteriorly 2 to

Nassarius velvetosus new species
(Figs. 10 and 11)

Type material

Holotype MNHN IM 2007-31709; BOLD ID = NEOGA 1187-12; GenBank accession number for COI = KC970064 (Fig. 10A–C) (height 22.0 mm, width 10.1 mm). Seven paratypes
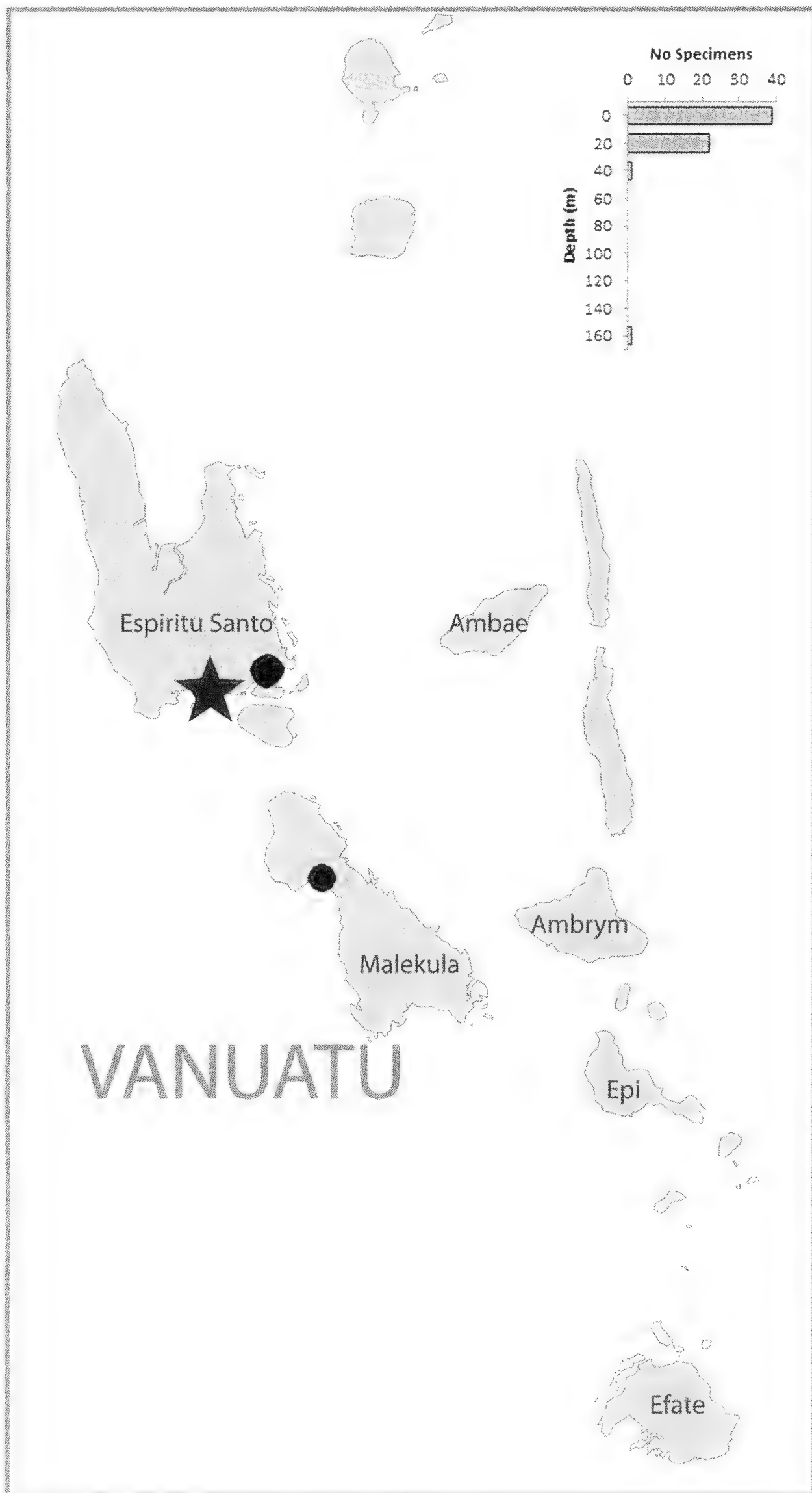
Figure 9. Geographical and bathymetrical distribution of *Nassarius vanuatuensis* sp. nov. Each bar represents all lv or dd specimens. Star indicates type locality.

3 pustules. Parietal denticle strong. Anal canal prominent. Siphonal area with 6 to 7 grooves.

Shell white, scattered with brown, body whorl on dorsal side reddish-brown, darker near suture, lighter near outer lip with 2 faint bands. Aperture, outer lip and columellar callus white. Operculum (in paratype) yellowish, serrated.

After photography the protoconch and the anterior part of the outer lip of the holotype have been damaged.

Intraspecific variation: There is a rather great difference in color. Dead-collected shells lose the brown color and become

white, which hinders an accurate identification. Size of adult specimens varies from 15.1 to 25.5 mm.

Distribution

Nassarius velvetosus sp. nov. lives between 24–302 m from Vanuatu to New Caledonia. The occurrence in Fiji has to be confirmed with better quality additional specimens (Fig. 11).

Discussion

Some of the lots used here to describe *N. velvetosus* sp. nov. have already been mentioned by Cernohorsky (1991: 200), who misidentified them with *Nassarius comptus* (A. Adams, 1852). Specimens resembling *N. comptus* are frequently misidentified. However, the lectotype of *N. comptus* (Fig. 7A–D) shows strongly ribbed early whorls, whereas *N. velvetosus* sp. nov. has many fine ribs on the first 3 teleoconch whorls and is considerably more slender.

Cernohorsky (1991: 200) also misidentified another lot of *N. velvetosus* sp. nov. under the name *Nassarius haldemanni* (Dunker, 1847) (Fig. 12A–C). Dunker (1847: 62) described the species with the name "*Buccinum Haldemani*" with a single 'n', evidently after the American malacologist Samuel Haldeman (1812–1880), although no etymology was given. The spelling *Nassarius haldemanni*, frequently used in literature, is a misspelling. The holotype of *N. haldemani*, figured by Cernohorsky (1984: 283, Fig. 10), is considerably smaller in size (up to 14 mm) and has a smooth, carinated protoconch of 3 whorls; protoconch and first teleoconch whorl brownish.

A third resembling species is *Nassarius tangaroai* Kool, 2006 (Fig. 12D–F), which is smaller in size and has fewer and stronger ribs on the first apical whorls. It is known only from the Marquesas Archipelago.

Etymology

Velvetosus, Latinization of velvety. The velvety sheen of the shell inspired the name assignation.

***Nassarius martinezi* new species**
(Figs. 13 and 14)

Type material

Holotype MNHN IM 2007-35021; BOLD ID = NASSA 005-12; GenBank accession number for COI = KC970038 (Fig. 13A–C, H) (height 18.9 mm, width 8.8 mm). Thirteen paratypes MNHN, 1 paratype HK 172.02: New Caledonia, Munida Bank, NORFOLK 2, stn CP2139, 23°01′S, 168°23′E, 372–393 m, 1 lv, height 19.6 mm, width 9.5 mm (IM 2007-35031) (Fig. 13D–G); 1 lv (MNHN IM 2007-35030); 1 lv (MNHN IM
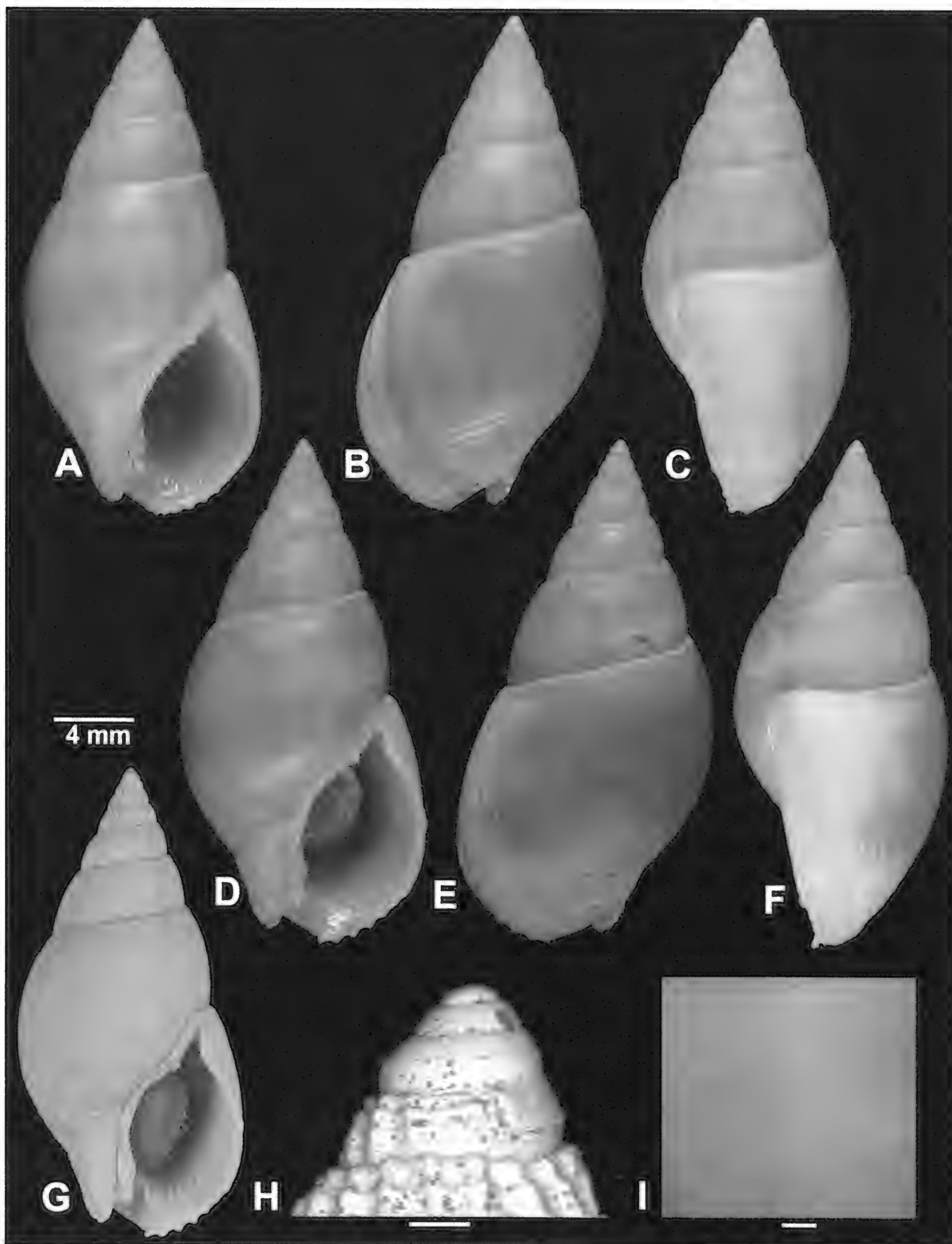
Figure 10. *Nassarius velvetosus,* sp. nov. **A–C,** holotype MNHN IM 2007-31709, h 22.0 mm, w 10.1 mm, Vanuatu, South Urelapa Island, SANTO 2006: stn EN25, 15°37′S, 167°02′E, 116 m. **D–G,** paratype MNHN IM 2009-13088, h 22.8 mm, w 10.3 mm, Vanuatu, South Urelapa Island, SANTO 2006: stn EN20, 15°37′S, 167°02′E, 85 m. **H,** protoconch of paratype. **I,** Enlarged shell sculpture. Scale bar: H and I = 250 μm.

Grand Passage, CONCALIS, stn CP3008, 18°30′S, 163°04′E, 275–305 m, 1 lv (MNHN IM 2007-34766); New Caledonia, Grand Passage, CONCALIS, stn DW2986, 17°59′S, 163°06′E, 270–300 m, 1 lv (MNHN IM 2007-34768); New Caledonia, PALEO-SURPRISE, stn DW1391, 18°30′S 163°03′E, 365 m, 1 dd (MNHN IM 2000-25567).

Type locality

New Caledonia, Norfolk Ridge, Munida Bank, 23°02′S, 168°21′E, 295–330 m [NORFOLK 2, stn DW2135].

Additional material (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0202/suppl_file/Kool_2014_Suppl.pdf)

Description

Shell elongate, ovate, height 18.9 mm, width 8.8 mm, extremely thin, shining. Planktotrophic protoconch with approximately 3 carinated whorls (Fig. 13H). Teleoconch with 5 whorls with numerous narrow, flat, equally-sized axial ribs all over the shell, crossed by numerous, even so low spiral ribs, resulting in square to oblong interstices (Fig. 13B, I). Suture prominently ledged, with a subsutural row of fine granules.

Aperture oval, outer lip thin, edge somewhat turned backwards, anteriorly with 5–6 fine blunt denticles. Columella smooth and shiny. Columellar callus thin and passing into a thin and glazy enamel on part of ventral side of last whorl, nearly up to the suture. Parietal denticle nearly absent, anal canal wide. Siphonal area with eight ribs.

Shell white, with yellowish-brownish tinge all over the shell, except on the first 1–2 teleoconch whorls, the outer lip, the siphonal canal and the columella.

Variation: Color varies among different shades of brown. Some specimens have a white spire and yellow bands on the

2007-35032); 1 lv (MNHN IM 2009-12252); 1 lv (MNHN IM 2009-12253); 1 lv (MNHN IM 2009-12254); 1 lv (MNHN IM 2009-12255); 1 lv (MNHN IM 2009-12256), 1 lv (MNHN IM 2009-12243); 1 dd HK 172.02; New Caledonia, Norfolk Ridge, Munida Bank, TERRASSES, stn DW3108, 23°01′S, 168°23′E, 370–440 m, 1 lv (MNHN IM 2007-36807); New Caledonia,
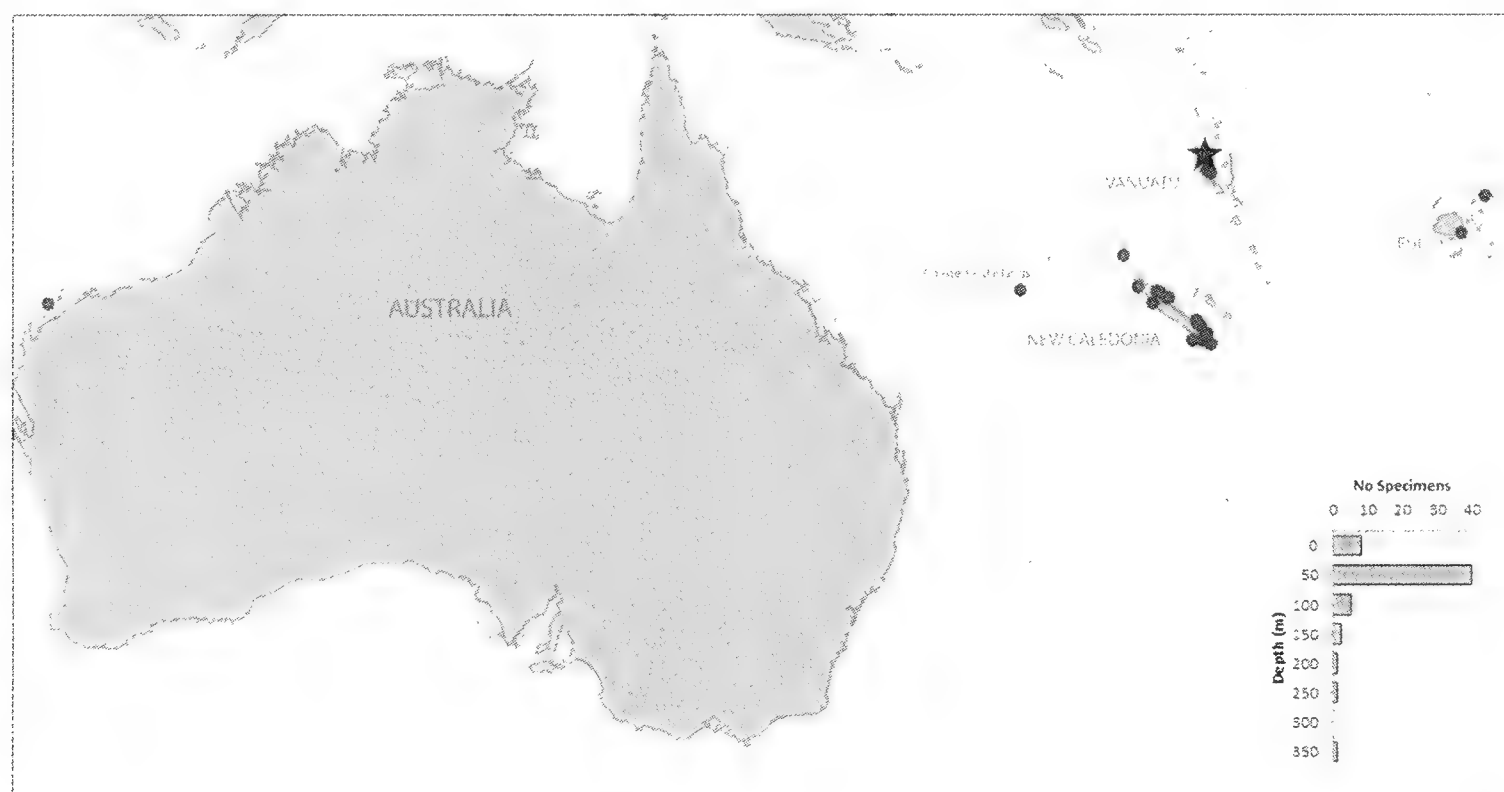
Figure 11. Geographical and bathymetrical distribution of *Nassarius velvetosus* sp. nov. Each bar represents all lv or dd specimens. Star indicates type locality.

dorsal part of the last whorl. Some specimens are deeper channeled or have fewer axial and spiral ribs on the last whorl. Size from 11.5 to 20.0 mm.

Distribution

The species lives at depths of 168–372 m, from New Caledonia to the Solomon Islands, Fiji and Tonga (Fig. 14).

Discussion

The combination of sculpture and fragility of the shell are species-specific in comparison with other nassariid species. The shell of *N. houbricki* sp. nov. is similar in shape but larger in size, thicker, with stronger axial ribs. *Nassarius alabasteroides* is larger and more elongate than *N. martinezi* sp. nov. which has a thinner shell. The sculpture of *N. noguchii* Habe, 1958 is somewhat comparable with that of *N. martinezi* sp. nov., but the shell of the former is smaller, not as thin and less fragile.

Etymology

Named in honor to Rafael "Guru" Martínez, Curator of the Mollusca Collection of the Central University of Venezuela who is considered to be the father of malacology in Venezuela after 50 years of research in the Caribbean Sea and teaching many marine biology students, including the second author of this paper.

Molecular section

We used COI sequences to support our species delimitation hypotheses primarily based in morphological recognition. Ninety one partial sequences of 658 bp were unambiguously aligned without gaps. Two hundred fifty one were found to be variable positions of which 229 were parsimony-informative.

The intra-specific genetic variability among the *Nassarius* included in present study is between 0 and 0.019 (Fig. 15). The results agree with what has been found for different gastropod species. For instance, among nassariids the intra-specific variation varies between 1% within *Nassarius reticulatus* (Linnaeus, 1758) (Couceiro *et al.* 2007), 1.1% within *Nassarius nitidus* (Jeffreys, 1867) (Couceiro *et al.* 2012) and 2.5% for *Cyplope neritea* (Linnaeus, 1758) (Simon-Bouhet *et al.* 2006). Barcode gap is 2.5% in different genera of Conoidea (Puillandre *et al.* 2009, 2010). Barco *et al.* (2013) has reported comparable results for intra-specific variability among the genus *Gibbula* Risso, 1826 (0–0.278).

The taxonomic tree is presented in Figure 16. *Nassarius ocellatus* sp. nov. appears as a monophyletic group and distinct from *N. praecallosus* (Marrat, 1877), its sister-species. Sequences of *N. multigranosus* are not available for molecular comparison. K2P Genetic distances between the two species span from 4.2 to 5.9%, similar to those distances generally found between different gastropod species (Li *et al.* 2010, Kantor *et al.* 2012).

Comparison between sequences of *N. houbricki* sp. nov. and *N. siquijorensis* reveals 6.2 to 7.1% genetic divergences. Sequences of *N. alabasteroides* and *N. psila* are not available. The closest molecular species is another undescribed species, with genetic distances to *N. houbricki* sp. nov. comprised between 4.4 to 5.5%

Comparisons of genetic distances between *N. radians* sp. nov. with *N. thachi* (5.3–5.6%), *N.* cf. *comptus* (7.4–8.7%), *N.* cf. *cinnamomea* (6.7–7.8%) and *N. haldemani* (8.5–8.9%) support our species delimitation. *N. acuminatus* (Marrat, 1880) is the closest molecular species to *N. radians* sp. nov., with genetic distances between 3.5 and 3.8%. COI sequences are not available for *N. nanhaiensis*.

Although morphologically similar to *N. pauper* (genetic distances between 19.5 and 19.9%), *Nassarius vanuatuensis* sp. nov. does not belong to the *pauper* complex as discussed in Kool and Dekker (2006, 2007) (Fig. 16). The closest species is *N. haldemani* with genetic distances from 9.4 to 9.6%. No COI sequence is available for *Nassarius moestus*.

The differences in terms of genetic distances between *Nassarius velvetosus* sp. nov. and *N. cinnamomea* (7.8–9.5%), *N.* cf. *comptus* (7.7–9.4%) and *N. barsdelli* Ladd, 1976 (4.6–4.8%) support our species hypothesis. Comparison with *N. tangaroai* was not posible. *N. velvetosus* sp. nov. is related to a not yet described species (with distances of 5.6–6.4%).

Although the comparison with *N. alabasteroides* was not possible, sequences of *N. martinezi* sp. nov. were different enough from those of *N. houbricki* sp. nov. (genetic distances between 6.8 and 7.4 %) and *N. noguchii* (4.4–5.0%) to
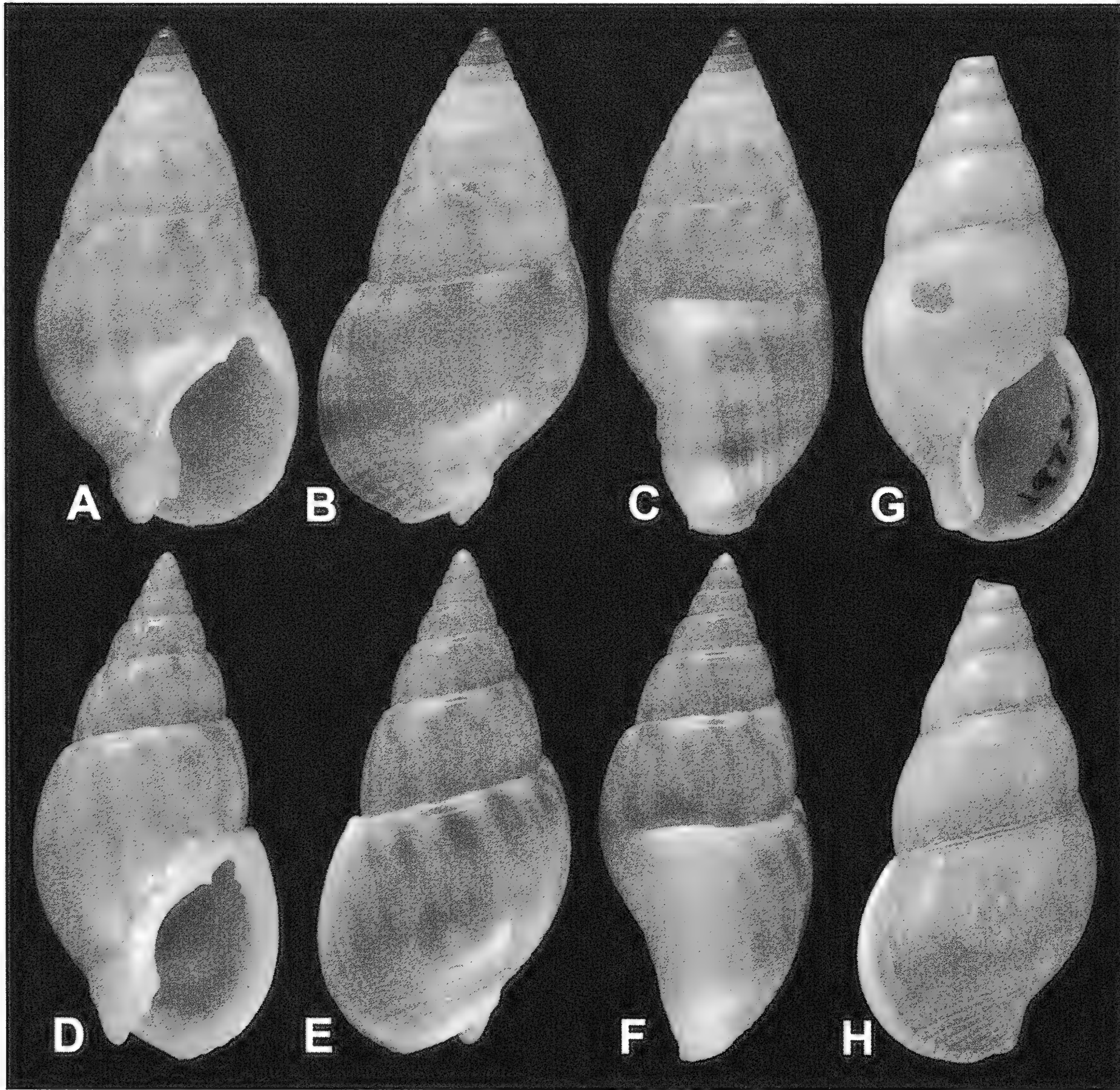
Figure 12. *Nassarius haldemani* (Dunker, 1847). **A–C,** h 12.9, w 6.3 mm (HK 874.04), Indonesia, Celebes Sea, N. Sulawesi, near Pulau Balabangan Group, 02°26′S, 117°25′E, 5–25 m. *Nassarius tangaroai* Kool 2006. **D–F,** holotype MNHN 9920, h 16.7, w 7.4 mm Marquesas Archipelago, MUSORSTOM 9: stn DW1224, 9°45′S, 138°51′W, 115–120 m. *Nassa cinnamomea* A. Adams. **G–H,** holotype NHMUK 1973021, h 23.8 mm, w 11.4 mm, Philippines, Dumaguete, Negros Island.

Nassarius pauper (Gould, 1850), see Kool and Dekker 2006, 2007), which might lead to merge together closely related species under the same name (*e.g., Nassarius comptus* (A. Adams, 1852), see the discussion under *Nassarius radians* sp. nov.), with numerous misidentifications and sometimes long lists of synonyms (Cernohorsky 1984).

Characters informative for nassariid species recognition are currently almost exclusively based on shell features. Cernohorsky (1984) and Haasl (2000) discussed those characters and their limits (some subgenera could be identified by the radula) suggesting the need for further sources of data. To traditional taxonomic practices, the description of new nassariids has lately been confirmed by different molecular approaches. Studies on cryptic *Nassarius* species COI and 16S fragments (Li *et al.* 2010, Couceiro *et al.* 2007, 2012, Chen and Zhang 2012) have already been used. The main limitation of molecular approach is the lack of a robust dataset for comparison among monophyletic groups.

The ranges of intra- and interspecific genetic distances calculated for our new species, are congruent with the usage of barcode gap in

support our species delimitation. The other two closest related taxa are *N. haldemani* and an undescribed species, the genetic distances of which range between 8.0–8.6 and 3.3–3.9%, respectively.

DISCUSSION

Considering the relative abundance and the ecological importance of the species belonging to the family Nassariidae, the taxonomic status of many species remains uncertain. Within nassariids, name assignation represents a difficult challenge due to the fact that genera and species delimitation can be confusing in many cases. Species may have small morphological inter-specific differences (*e.g.,* the group of

nassariids (Simon-Bouhet *et al.* 2006, Couceiro *et al.* 2007, 2012, Li *et al.* 2010). For all the species described, the range of values of intraspecific divergence and the Bayesian tree confirmed the morphological discrimination made *a priori*. Even more, our threshold represent a value similar to those often found among gastropods (Puillandre *et al.* 2009, Kantor *et al.* 2012, Barco *et al.* 2013), reaffirming the utility of the DNA barcode to test species hypotheses delimitation.

We chose name-bearing types that are also hologenophores, to avoid the perpetuation of a molluscan taxonomy where many holotypes do not fulfill their function of name bearers (Bouchet and Strong 2010). The sequences obtained from the specimens support our taxonomic hypothesis concerning the new species formulated from morphological observations.
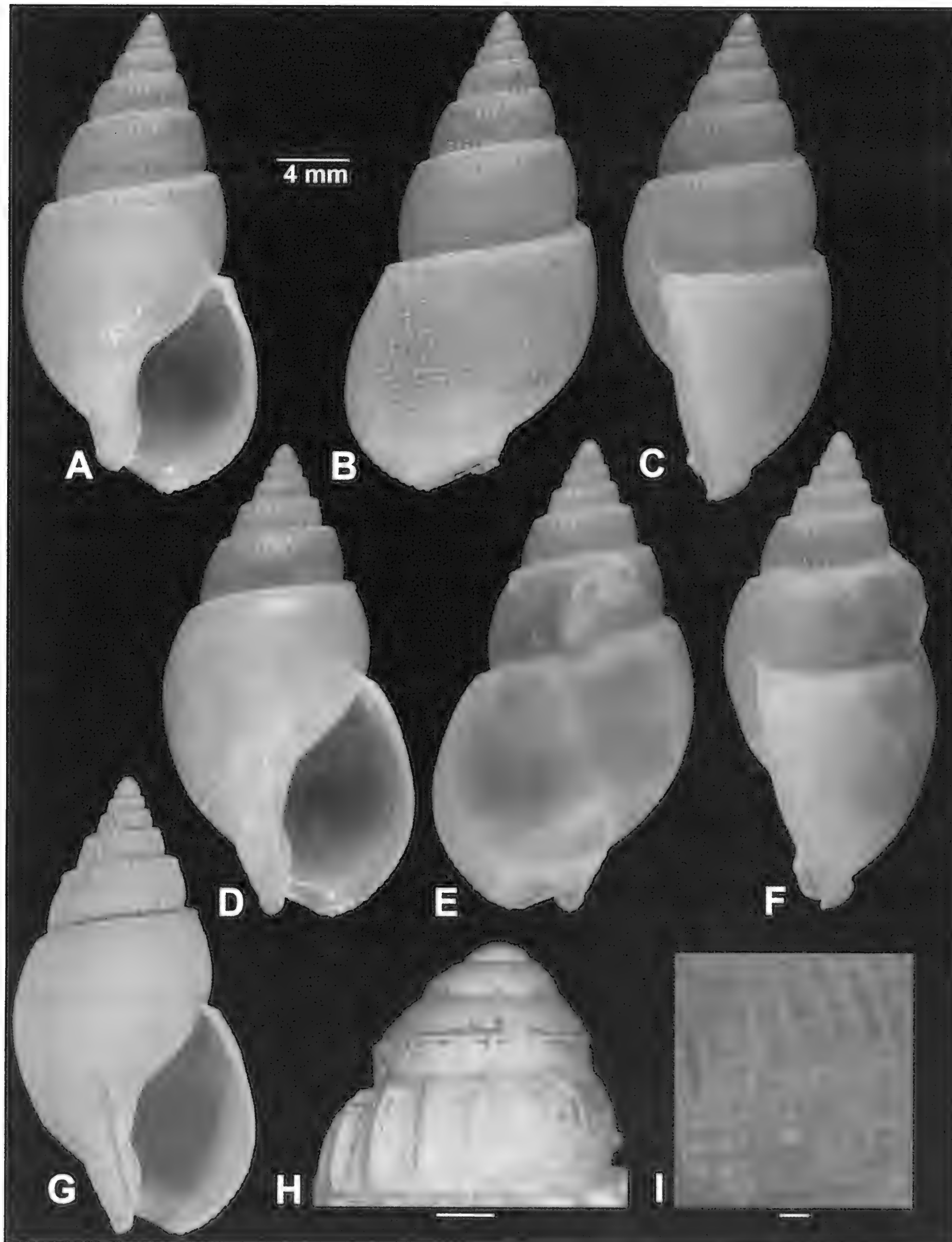
Figure 13. *Nassarius martinezi* sp. nov. **A–C**, holotype MNHN IM 2007-35021, h 18.9 mm, w 8.8 mm, New Caledonia, Munida Bank, NORFOLK 2: stn DW2135 23°02′S, 168°21′E, 295–330 m. **D–G**, paratype MNHN IM 2007-35031, h 19.6 mm, w 9.5 mm, New Caledonia, Munida Bank, NORFOLK 2: stn CP2139, 23°01′S, 168°23′E, 372–393 m. **H**, protoconch of the holotype. **I**, Enlarged shell sculpture. Scale bars: H and I = 250 μm.

ACKNOWLEDGMENTS

Figure 14. Geographical and bathymetrical distribution of *Nassarius martinezi* sp. nov. Each bar represents all lv or dd specimens. Star indicates type locality.

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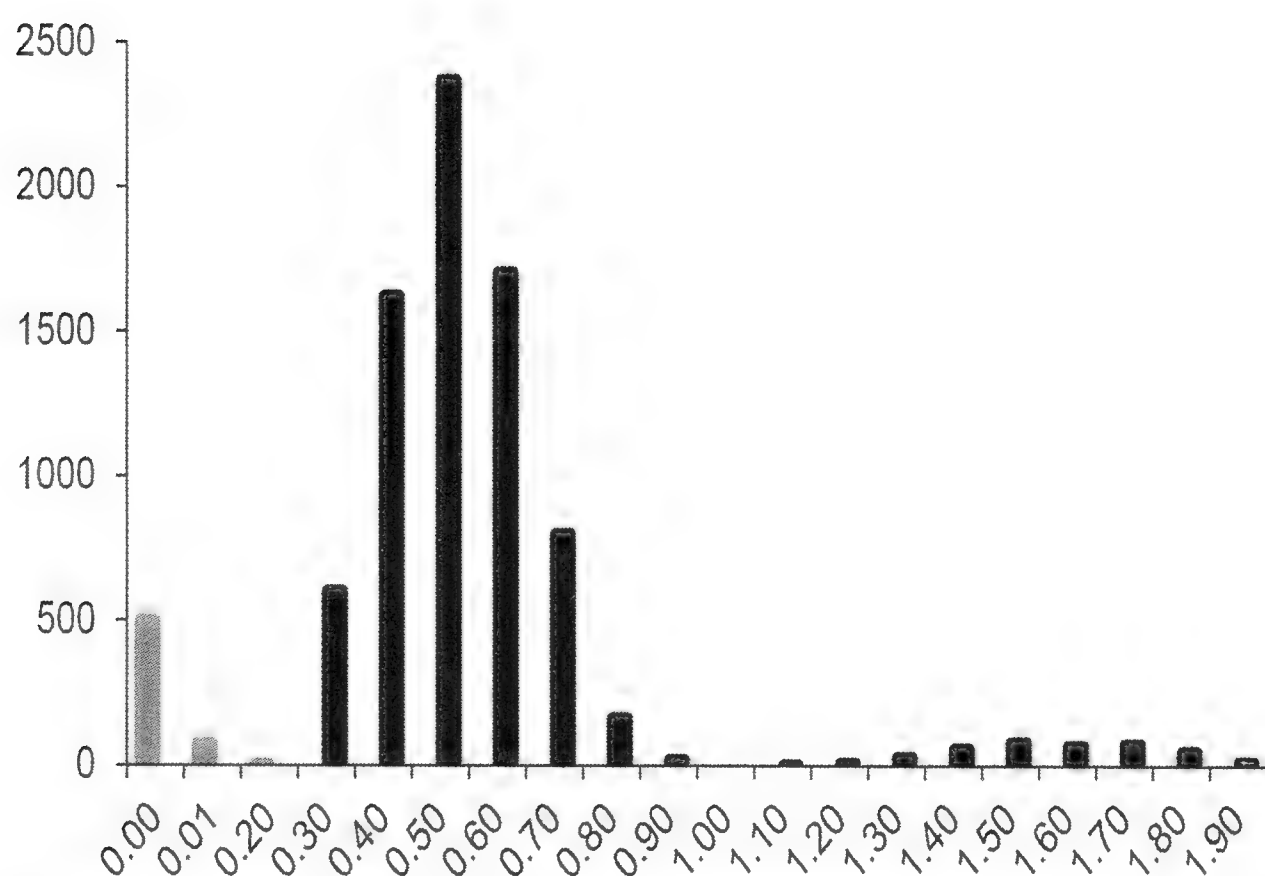
Figure 15. Frequency of pairwise distance (K2P model) values among COI sequences. In gray, intra-specific variation. In black interspecific variation.

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Two species of the genus *Strobiligera* (Caenogastropoda: Triphoridae) with a multispiral protoconch in Southeastern Brazil

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Abstract: The genus *Strobiligera* Dall, 1924, sometimes treated as a synonym of *Inella* Bayle, 1879, included up to now species geographically restricted to the North Atlantic Ocean, usually occurring at great depths. The present study records the genus *Strobiligera* in the South Atlantic Ocean, with the recognition of two species in Southeastern Brazil. The shell of *Strobiligera delicata* sp. nov., which has a white teleoconch and a light brown protoconch, is very similar in general coloration to the shells of *Strobiligera georgiana* (Dall, 1927) comb. nov. and *Strobiligera indigena* (Dall, 1927) comb. nov., both from the southeastern coast of the United States. However, *S. delicata* differs from those species in shell shape, number of axial cords on the teleoconch and earlier development of the adapical spiral cord of the teleoconch. *Strobiligera inaudita* (Rolán and Lee, 2008) comb. nov., previously known from the southern coast of the United States, is diagnosed by a teleoconch with adapical and abapical spiral cords tinted with brown and white nodules, and by a completely white median spiral cord. *Strobiligera delicata*, *S. inaudita*, *S. georgiana* and *S. indigena* have multispiral protoconchs, similar to those of their congeneric species from the East Atlantic (Europe and North Africa) and Mediterranean. Other species of *Strobiligera* from the Caribbean and neighboring areas, including the type species *Triforis ibex* Dall, 1881, have a paucispiral protoconch. Hence, the generic allocation of species with a multispiral protoconch to *Strobiligera* cannot be considered definitive, and should be confirmed by subsequent anatomical studies of species with different modes of development.

Key words: taxonomy; Mollusca; *Inella* group; Atlantic Ocean; microgastropods

Triphoridae is a large family of marine microgastropods, comprised of sponge feeders (Marshall 1983). They are usually recognized by the presence of a left-coiled shell in most species, besides a large variation in radular morphology. Despite being much more diverse in the Indo-Pacific (Albano *et al.* 2011), several authors (*e.g.,* Rolán and Fernández-Garcés 2008) have also reported a high number of species in the Atlantic Ocean.

Our knowledge of Triphoridae in Brazil has recently increased, with the description of a deep-sea species from the Canopus Bank (Simone 2006), the review of the genus *Metaxia* Monterosato, 1884 (Fernandes and Pimenta 2011), and the study of Triphorinae from the Vitória-Trindade Seamount Chain (Fernandes *et al.* 2013). The number of reported species of Triphoridae from Brazil has been increased to 26, and many remain to be described or reported (Fernandes *et al.* 2013).

Following the synonymy of Iniforinae with Triphorinae by Marshall (1983), Triphoridae has two subfamilies: Metaxiinae, composed only of dextral species, and Triphorinae, which includes the sinistral species. However, Marshall (1983) created the concept of the "*Inella* group" in Triphorinae and suggested that it could subsequently be worthy of subfamily status. This group can be recognized by a tendency for simultaneous development of the three spiral cords of the teleoconch (although the adapical one is usually weaker), an embryonic shell with cruciform tubercles, and the presence of a smooth spiral zone on the larval shell (Marshall 1983). The latter two features are present only in species with a multispiral protoconch (indicative of planktotrophic development).

In the "*Inella* group", *Strobiligera* Dall, 1924 was considered to be either a valid genus (Bouchet 1985, Bouchet and Warén 1993) or a possible synonym of *Inella* Bayle, 1879 (Rolán and Fernández-Garcés 2008). Currently, *Strobiligera* contains five species (Dall 1924, Gofas 2013), all of which are restricted to the North Atlantic. These species have different types of larval development: those from the Mediterranean and East Atlantic (Europe and North Africa) are exclusively of the planktotrophic type (multispiral protoconch), whereas those recorded from the Caribbean and neighboring areas are of the lecitotrophic type (paucispiral protoconch). Based on the assumption that different modes of larval development, thus different types of protoconch, can coexist in the same genus (Bouchet 1990), Bouchet (1985) and Bouchet and Warén (1993) included species with a multispiral protoconch in *Strobiligera*, although the type species of the genus has a paucispiral protoconch. This study reports the discovery of two species of *Strobiligera*, both with multispiral protoconch, in the South Atlantic, more precisely in Southeastern Brazil.

MATERIALS AND METHODS

Taxonomic identifications were based on conchological comparisons using stereomicroscope and scanning electron microscope (SEM) images. The whorl-counting procedure was based on Leal (1991) and the distinction between embryonic shell and larval shell follows Fernandes *et al.* (2013). General terminology was based on Marshall (1983), except in relation to the spiral cords, as we prefer to designate them as adapical, median, abapical, and sutural cords. In the lists of material examined, the number of shells in each lot is indicated in square brackets. All material examined consisted of empty shells.

Abbreviations used: (AMS) Australian Museum, Sydney, Australia; (BGR) Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover, Germany; (BMSM) The Bailey-Matthews Shell Museum, Sanibel, Florida U.S.A.; (FLMNH) Florida Museum of Natural History, Gainesville, Florida U.S.A.; (HAB) "Projeto Habitats – Heterogeneidade Ambiental da Bacia de Campos" (Habitats Project), carried out by CENPES/PETRO-BRAS, Research Vessel Miss Emma McCall coll.; (IBUFRJ) Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; (MD55) French-Brazilian Expedition MD55, Research Vessel Marion-Dufresne coll., May-June/1987; (MNHN) Muséum national d'Histoire naturelle, Paris, France; (MNRJ) Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; (MORG) Museu Oceanográfico do Rio Grande, Rio Grande, Brazil; (MZSP/MZUSP) Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; (REVIZEE) "Programa de Avaliação do Potencial Sustentável de Recursos Vivos da Zona Econômica Exclusiva" (Program of Evaluation of the Sustainable Potential of Living Resources in the Exclusive Economic Zone), Central Score, carried out by Ministério do Meio Ambiente, Brazilian Government (REVIZEE C1, Oceanographic Ship Antares coll.); (USNM/NMNH) National Museum of Natural History, Washington, D.C., U.S.A.

RESULTS

Class Gastropoda Cuvier, 1795
Subclass Caenogastropoda Cox, 1960
Superfamily Triphoroidea Gray, 1847
Family Triphoridae Gray, 1847
Genus *Strobiligera* Dall, 1924

Diagnosis (adapted from Dall 1924; Bouchet 1985; Bouchet and Warén 1993): paucispiral or multispiral protoconch; paucispiral protoconch with the first whorl smooth and inflated, larger than subsequent whorl, which can have two spiral cords; multispiral protoconch with embryonic shell covered by cruciform or arrowhead-shaped granules, larval shell with two spiral cords and incomplete axial sculpture;

teleoconch whorls usually with three spiral cords, the adapical one initially smallest but increasing in prominence throughout the teleoconch, rarely with only two evident spiral cords; radula with central, lateral and marginal teeth not differentiated, mainly with two elongated claw-shaped cusps.

Type species: *Triforis ibex* Dall, 1881. Original designation. Recent, Gulf of Mexico.

Strobiligera delicata Fernandes and Pimenta sp. nov.
(Figs. 1B–1K)

Type material: holotype: MNHN IM-2000-27528, MD55 sta. 54-CB93, 19°36′S, 38°53′W, 640 m, 30.v.1987. Paratypes: off Espírito Santo state: MNRJ 25955, MD55 sta. 41-DC73, 18°59′S, 37°48′W, 607–620 m, 27.v.1987 [1]; MNHN IM-2000-27529, MD55 sta. 43-CB77, 19°40′S, 37°48′W, 790–940 m, 27.v.1987 [3]; MZSP 114439, MD55 sta. 43-CB77, 19°40′S, 37°48′W, 790–940 m, 27.v.1987 [2]; off Rio de Janeiro state: MNRJ 25956, MD55 sta.65-CB106, 23°54′S, 42°10′W, 830 m, 02.vi.1987 [1].

Type locality: 19°36′S, 38°53′W, 640 m, off Espírito Santo state, Brazil.

Diagnosis: conical-fusiform shell; 3–3.5 protoconch whorls; adapical spiral cord of teleoconch becomes distinctive and nodulose at approximately the fourth or fifth whorl, reaching almost the same size as the abapical spiral cord between the eighth and tenth whorl; light brown protoconch, white teleoconch.

Description: shell sinistral, slender, narrow, conical-fusiform, rectilinear in profile, attaining up to 5.77 mm in length, 1.15 mm in width. Protoconch columnar, 0.40–0.50 mm in length, 0.34–0.39 mm in width, 3–3.5 convex whorls; embryonic shell dome-shaped and covered with several arrowhead-shaped granules; larval shell with two spiral cords intersected initially by granules, which gradually develop into axial ribs, interrupted at the boundary of the smooth spiral zone immediately above the adapical spiral cord; ~ 40 slightly sigmoid and discontinuous axial ribs. Teleoconch with up to 13 whorls; two main spiral cords (median and abapical) at the beginning, derived from the protoconch spiral cords, and a very small one (adapical) above, close to the median cord; adapical spiral cord gradually strengthens, becoming distinctive and nodulose at approximately the fourth or fifth whorl, reaching almost the same size as the abapical spiral cord between the eighth and tenth whorl; median spiral cord slightly more pronounced than the others; 15–18 narrow and slightly opisthocline axial ribs on the seventh whorl of the teleoconch; nodules of a medium size, almost round, partially cut by the spiral cords, mainly on median and abapical ones; shallow

but distinctive suture, with a small sutural cord; smooth sub-peripheral cord and two smooth basal cords, the abapical one very small; absence of supranumerical spiral cords at the end of the body whorl; aperture ovoid; anterior and posterior canals broken or not formed; thick inner lip. Light brown protoconch, white teleoconch.

Geographic distribution: Brazil: off Espírito Santo and Rio de Janeiro states.

Bathymetric distribution: 607–940 m.

Remarks: *Strobiligera delicata* sp. nov. is very similar to *Strobiligera georgiana* (Dall, 1927) comb. nov. and *Strobiligera indigena* (Dall, 1927) comb. nov., two species that are known only from Georgia, U.S.A. The lectotype of *S. georgiana* was designated by Rolán and Fernández-Garcés (2008) and it is illustrated herein (Fig. 1A) because this is the most similar species to *S. delicata*. The three species show the same color pattern—a white teleoconch with a beige/brown protoconch—in addition to slow development of the adapical spiral cord of the teleoconch and a rectilinear profile of the shell. The bathymetric distribution of *S. delicata* (607–940 m depth) overlaps with that of *S. georgiana* and *S. indigena*, which were recorded at 805 m depth. Unfortunately, the protoconchs of the type material of *S. georgiana* and *S. indigena* are at least partially eroded (Rolán and Fernández-Garcés 2008), hindering a detailed comparison with *S. delicata*, particularly on the embryonic shell. Dall (1927) described the embryonic shell of *S. georgiana* as being smooth (probably because it was worn) and the larval shell as possessing two spiral cords and "numerous retroactively [interrupted?] flexuous axial threadlets", just like in *S. indigena*, which coincides with the larval shell of *S. delicata* (Fig. 1I). Because of these similarities, we also consider *S. georgiana* and *S. indigena*, species of the West Atlantic with a multispiral protoconch, as representatives of *Strobiligera* (Table 1).

Compared to *Strobiligera delicata*, *S. georgiana* has a more conical shell, a later development of the adapical spiral cord and a reduced number of axial cords on the teleoconch (~ 12 axial cords on the seventh whorl of the lectotype of *S. georgiana*: Fig. 1A; 15–18 axial cords on the same whorl on *S. delicata*: Figs. 1B–1C). Compared to the lectotype of *Strobiligera indigena* (illustrated by Rolán and Fernández-Garcés 2008), *S. delicata* shows earlier development of the adapical spiral cord and less convex whorls on the teleoconch (Figs. 1B–1C). Also, *S. delicata* reaches up to 5.77 mm in length at 13 whorls of teleoconch (protoconch complete), which is considerably less than *S. georgiana* (lectotype measuring 7.5 mm at 13 whorls of teleoconch, protoconch incomplete) and *S. indigena* (lectotype measuring 6.6 mm at 11 whorls of teleoconch, apex missing).

Triphora caracca Dall, 1927, from Georgia (U.S.A), also shows the same coloration as *Strobiligera delicata*. However, *T. caracca* can be easily distinguished on the basis that its median spiral cord develops later, on the tenth whorl (Rolán and Fernández-Garcés 2008), a feature that makes it more similar to the genus *Monophorus* Grillo, 1877.

Etymology: the name of this species alludes to the delicate aspect of its shell.

Strobiligera inaudita (Rolán and Lee, 2008) comb. nov.
(Figs. 2A–2K)

Triphora sp.: Absalão *et al.* (2006: 238, *in part*).
Triphora inaudita Rolán and Lee *in* Rolán and Fernández-Garcés (2008: 150, figs. 26 A–D); García and Lee (2011).

Type material: holotype: FLMNH 419185. Paratype: BMSM 15203.

Type locality: 26°42.9′N, 83°43.2′O, 73.3–78.5 m, Dry Tortugas, Florida, U.S.A.

Material examined: the holotype, and, additionally, from Brazil: off Espírito Santo state: IBUFRJ 19504, REVIZEE C1 sta. VV38, 19°28′26″S,38°22′30″W, 71 m, 29.ii.1996 [1]; IBUFRJ 19613, REVIZEE C1 sta. VV38, 19°28′26″S,38°22′30″W, 71 m, 29.ii.1996 [1]; Campos Basin (off Espírito Santo and Rio de Janeiro states): IBUFRJ 19523, 20°47′S,40°26′W, Oc. ship Almirante Câmara coll., 29.viii.1979 [1]; IBUFRJ 19592, REVIZEE C1 sta. D3, 22°04′30″S,40°04′55″W, 98 m, 13.ii.1996 [1]; MNRJ 19478, 22°42′S,40°40′W, 110 m, 11.iv.2003 [4]; MNRJ 30870, 22°42′S,40°40′W, 110 m, 19.ix.2003 [1]; MNRJ 30905, 22°42′S,40°40′W, 110 m, 19.ix.2003 [1]; MNRJ 31106, 22°42′S,40°40′W, xi.2007 [1]; MNRJ 31125, 22°42′S,40°40′W, xii.2001 [1]; MNRJ 32348, 22°42′S,40°40′W, 110 m, ix.2004 [3]; IBUFRJ 11695, 22°48′S,40°45′W, 110 m, supply boat N/RB Astro Garoupa coll., 27.i.1998 [7]; IBUFRJ 19562, 22°48′S,40°45′W, 110 m, supply boat N/RB Astro Garoupa coll., iv.1998 [7]; MNRJ 18382, HAB 16 sta. C5-R1, 22°57′24″S,40°50′36″W, 142 m, 03.vii.2009 [1]; MNRJ 18971, 23°04′S,40°59′W, 17.xii.2004 [2]; MNRJ 18429, HAB 16 sta. B5-R3, 23°12′04″S,40°59′42″W, 142 m, 02.vii.2009 [1]; MORG 40691, Campos Basin [1]; MORG 40730, Campos Basin [1]; MORG 52230, Campos Basin, 2003 [1]; MORG 52234, Campos Basin, 2003 [1]; MORG 52237, Campos Basin [1]; MORG 52242, Campos Basin, 2003 [1].

Characterization: shell sinistral, slender, narrow, conical-fusiform, rectilinear in profile, attaining up to 8.00 mm in length, 1.80 mm in width. Protoconch columnar, 0.39–0.45 mm in length, 0.31–0.33 mm in width, 3.5–4 convex whorls; embryonic shell dome-shaped and covered with several arrowhead-shaped granules; larval shell with two
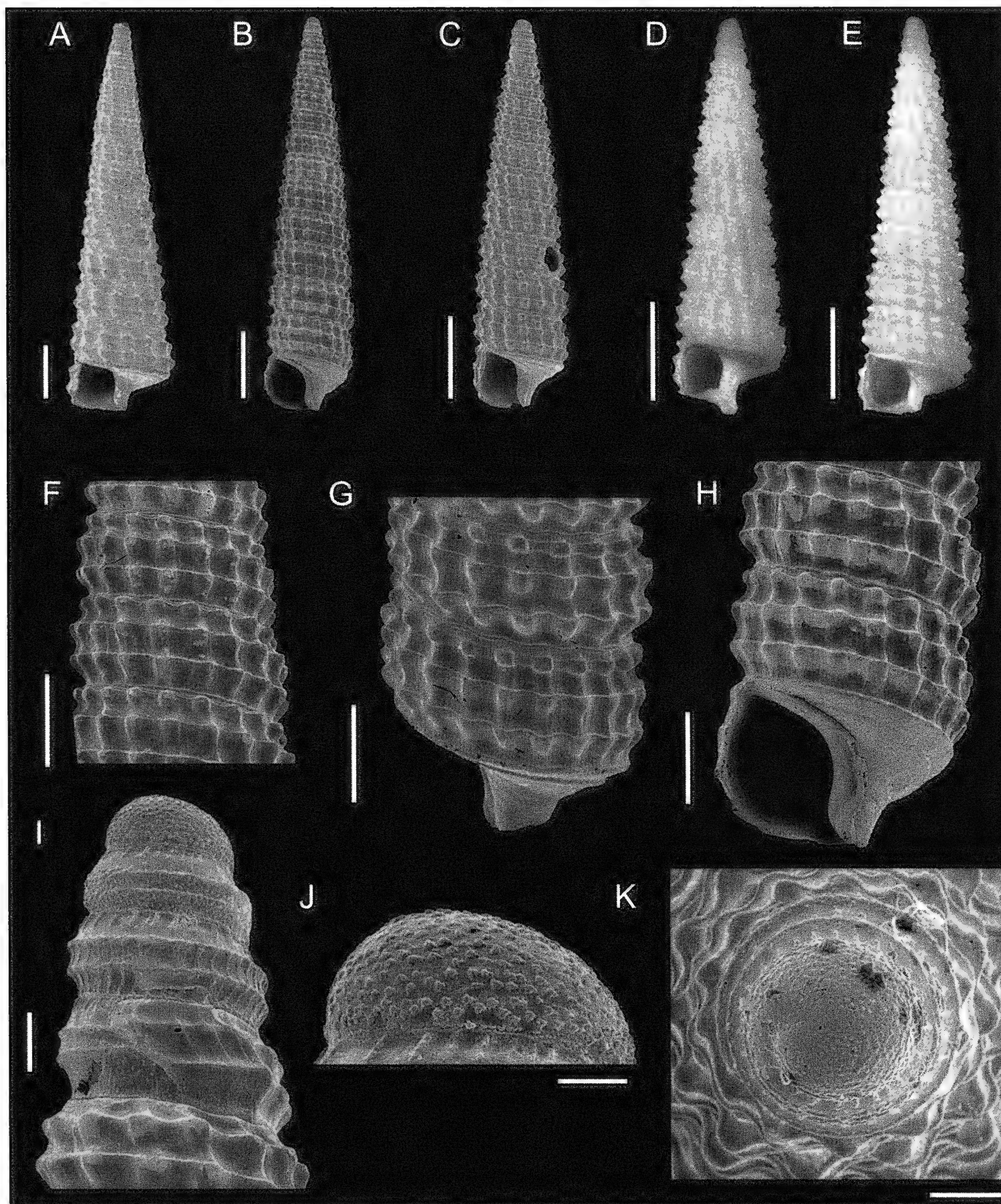
Figure 1. **A,** *Strobiligera georgiana* comb. nov. **B–K,** *Strobiligera delicata* sp. nov.; **A–E,** Entire shell, frontal view; **F,** detail of the teleoconch; **G,** abapical portion of the shell, dorsal view; **H,** abapical portion of the shell, frontal view; **I,** protoconch and initial portion of the teleoconch; **J,** embryonic whorl; and **K,** protoconch, adapical view. A, Lectotype USNM 333516; B, F, H–J, Holotype MNHN IM-2000-27528; C, G, K, Paratype MNRJ 25955. D–E, paratypes MNHN IM-2000-27529. Scale bars: A–E, 1 mm; F–H, 500 μm; I, K, 100 μm; J, 50 μm. (Color shown in electronic version only).

orthocline axial cords on the seventh whorl of the teleoconch; nodules of a medium size, rounded (particularly on the adapical spiral cord) to elliptic (particularly on the abapical spiral cord); shallow but distinctive suture, with a small sutural cord; weakly nodulose subperipheral cord, wavy adapical basal cord, slightly wavy to smooth abapical basal cord; supranumerical spiral cord at the end of the body whorl, between median and abapical spiral cords; oval aperture; short and open anterior canal, but crossed at its base by a projection of the outer lip; narrow and deep posterior canal, almost detached from aperture; thick inner lip. Light brown protoconch; teleoconch with the two initial whorls white; remaining whorls with a white median spiral cord, adapical and abapical spiral cords usually with one or two orange-brownish nodules intercalated with one or two white nodules; orange-brownish base.

Geographic distribution: U.S.A.: Florida (type locality), Louisiana (Rolán and Fernández-Garcés 2008); Brazil: off Espírito Santo and Rio de Janeiro states (present study).

Bathymetric distribution: 58 m (Rolán and Fernández-Garcés 2008)– 142 m (present study).

Remarks: the holotype of *Strobiligera inaudita* (Fig. 2A) was examined, but with the shell already partially broken (protoconch detached from teleoconch). *Strobiligera inaudita* is easily distinguished from its congeners due to the radial coloration pattern of its teleoconch (Figs. 2A–2C), whereas other species of *Strobiligera* usually have a homogeneously colored teleoconch. *Strobiligera flammulata* Bouchet and Warén, 1993, from the Northeast Atlantic and Mediterranean, has an axial pattern of coloration, with brown blotches on the three spiral cords, rather than a white median spiral cord as in *S. inaudita*.

Despite being represented in collections only by empty shells, *Strobiligera inaudita* seems to be restricted to deeper layers of the continental shelf from 58–142 m (Rolán and Fernández-Garcés 2008; Garcia and Lee 2011; present study).

spiral cords intersected initially by granules, which gradually develop into axial ribs, interrupted at the boundary of the smooth spiral zone immediately above the adapical spiral cord; ~ 40 discontinuous axial ribs that vary from almost rectilinear to slightly sigmoid. Teleoconch with up to 15 whorls; two main spiral cords (median and abapical) at the beginning, derived from the protoconch spiral cords, and a very small one (adapical) above, close to the median cord; adapical spiral cord gradually strengthens, reaching the same size as the others between the ninth and tenth whorls; 16–18 nearly

Table 1. Updated list of species assigned to *Strobiligera*.

Species	Protoconch	Geographic distribution	Bathymetric distribution
Strobiligera brychia (Bouchet and Guillemot, 1978)	Multispiral	Northeast Atlantic, Mediterranean*	140–2220 m*
Strobiligera lubrica Bouchet and Warén, 1993	Multispiral	Northeast Atlantic*	2000 m*
Strobiligera flammulata Bouchet and Warén, 1993	Multispiral	Northeast Atlantic, Mediterranean*	75–545 m*
Strobiligera delicata sp. nov.	Multispiral	Southeastern Brazil**	607–940 m**
Strobiligera inaudita (Rolán and Lee, 2008) comb. nov.	Multispiral	Southern U.S.A., Southeastern Brazil**	58–142 m**
Strobiligera georgiana (Dall, 1927) comb. nov.	Multispiral	Georgia (U.S.A.)***	805 m***
Strobiligera indigena (Dall, 1927) comb. nov.	Multispiral	Georgia (U.S.A.)***	805 m***
Strobiligera ibex (Dall, 1881)	Paucispiral	Gulf of Mexico***	823–1189 m***
Strobiligera bigemma (Watson, 1880)	Paucispiral	Culebra island (Puerto Rico)***	713 m***
Strobiligera inflata (Watson, 1880)	Paucispiral	Culebra island (Puerto Rico)***	713 m***
Strobiligera compsa (Dall, 1927) comb. nov.	Paucispiral	Georgia (U.S.A.)***	805 m***
Strobiligera enopla (Dall, 1927) comb. nov.	Paucispiral	Florida (U.S.A.) and Cuba***	538–823 m***
Strobiligera meteora (Dall, 1927) comb. nov.	Paucispiral	Florida (U.S.A.)***	538 m***
Strobiligera pompona (Dall, 1927) comb. nov.	Paucispiral	Georgia (U.S.A.)***	805 m***
Strobiligera dinea (Dall, 1927) comb. nov.	Paucispiral	Georgia (U.S.A.)***	805 m***
Strobiligera sentoma (Dall, 1927) comb. nov.	Paucispiral	Florida (U.S.A.)***	538 m***
Strobiligera gaesona (Dall, 1927) comb. nov.	Paucispiral	Georgia (U.S.A.)***	805 m***

* based on Bouchet and Warén (1993)
** based on the present study
*** based on Dall (1927) and Rolán and Fernández-Garcés (2008)

The upper limit of this bathymetric range basically corresponds to the supposed limit of 60 m below which initiates the twilight zone (Pyle 2001). This limit marks an apparent transition in the species composition of triphorids (Albano *et al.* 2011), more or less related to specific kinds of sponges, which are their source of food.

The discontinuous geographic distribution of *Strobiligera inaudita*, with isolated records from Southern U.S.A. and Southeastern Brazil, and apparent absence from the Caribbean, is probably due to its recent description and the reduced number of dredgings at the bathymetric range of this species.

DISCUSSION

The type species of *Strobiligera*, *Strobiligera ibex* (Dall, 1881), was described as having a paucispiral protoconch with a smooth and inflated first whorl that is much larger than the subsequent whorl [these features were cited by Dall (1924) in his description of *Strobiligera* as a subgenus of *Triphora* Blainville, 1828]. This type of protoconch (Fig. 3A) is very different from that of the type species of *Inella*, *Inella gigas* (Hinds, 1843), which has a distinctive paucispiral protoconch with well-defined spiral cords and no axial sculpture (Marshall 1983: figs. 10 D, H); Fig. 3B shows a typical (in the sense of the type species) protoconch of *Inella*. For this reason, we agree with Bouchet (1985) and Bouchet and Warén (1993) in considering *Strobiligera* as a valid genus.

The embryonic shell of a multispiral protoconch in *Strobiligera* can be covered with cruciform granules, as in *Strobiligera flammulata*, or with arrowhead-shaped granules, as in *Strobiligera brychia* (Bouchet and Guillemot, 1978), *Strobiligera delicata* (Fig. 1J) and *Strobiligera inaudita* (Fig. 2J). The arrowhead-shaped granules appear to be a variation of the cruciform granules.
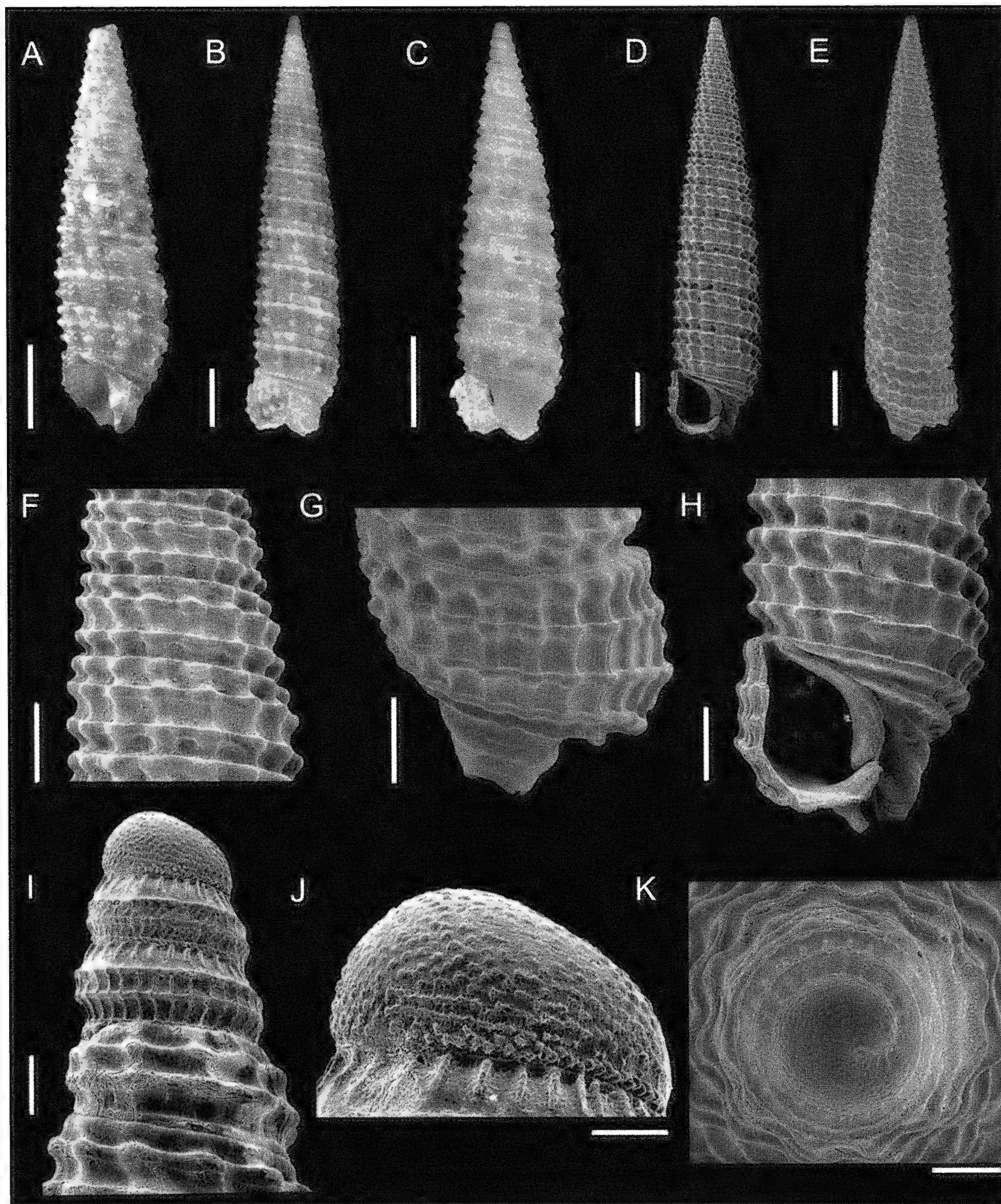
Figure 2. *Strobiligera inaudita* comb. nov. **A–D,** Entire shell, frontal view; **E,** entire shell, dorsal view; **F,** detail of the teleoconch; **G,** abapical portion of the shell, dorsal view; **H,** abapical portion of the shell, frontal view; **I,** protoconch and initial portion of the teleoconch; **J,** embryonic whorl, and **K,** protoconch, adapical view. **A,** Holotype FLMNH 419185; **B,** IBUFRJ 19562; **C–K,** IBUFRJ 11695. Scale bars: A–E, 1 mm; F–H, 500 μm; I, K, 100 μm; J, 50 μm. (Color shown in electronic version only).

morphology, besides radular features of species with a multispiral protoconch (Bouchet 1985, Bouchet and Warén 1993). However, the inclusion of species with a multispiral protoconch in *Strobiligera* cannot be considered as definitive, as there are no available descriptions of soft parts of any *Strobiligera* species with a paucispiral protoconch (including *Strobiligera ibex*), thus precluding further comparisons. As it seems difficult to obtain radulae of *S. ibex* and its deep-sea congeneric species with a paucispiral protoconch, combined with the geographical proximity with those species possessing multispiral protoconch and herein also treated as *Strobiligera*, we prefer not to create a new genus to accommodate the species with multispiral protoconch, but to follow the broad concept of *Strobiligera* by Bouchet (1985) and Bouchet and Warén (1993).

In the present work, we update the number of species of *Strobiligera*, transferring some Caribbean species currently allocated to *Inella* and *Triphora* to this genus (Table 1). Partially, we followed Dall (1927), who considered some of these species as belonging to the theretofore subgenus *Strobiligera*. The records of *Strobiligera pompona* (Dall, 1927) comb. nov. and *Strobiligera compsa* (Dall, 1927) comb. nov. to Brazil [Rios (2009) and Absalão (1989), respectively] are misidentifications and do not belong in *Strobiligera* (M. R. Fernandes, pers. obs.). Their correct taxonomic allocation will be provided in a subsequent paper.

Strobiligera seems to be essentially from deep waters, as indicated by Dall (1924) and Bouchet (1985). The species occurring at the shallowest depths are *Strobiligera inaudita* (up to 58 m), *Strobiligera flammulata* (up to 75 m), and *Strobiligera brychia* (up to 140 m; however, living specimens have been recorded as deep as 850 m). The remaining species range from 538 m (*Strobiligera enopla* and *Strobiligera meteora*) to 2000 m (*Strobiligera lubrica*) (Table 1), indicating a major presence on the continental slope. Although most of these species are known only by empty shells, we discard the hypothesis of a generalized vertical distribution *post mortem* that would alter radically the original bathymetric range of all the 17 species in *Strobiligera* (Table 1).

Regarding the sculpture of the teleoconch, the type species *Strobiligera ibex* (illustrated in Rolán and Fernández-Garcés 2008) possesses two evident spiral cords. However, according to Dall (1924, 1927), the concept of *Strobiligera* mainly comprises species with three spiral cords [*e.g., Strobiligera dinea* (Dall, 1927) comb. nov.; *Strobiligera meteora* (Dall, 1927) comb. nov.], even when the adapical one is very small [*e.g., Strobiligera enopla* (Dall, 1927) comb. nov.; *Strobiligera pompona* (Dall, 1927) comb. nov.], thus resembling *S. ibex*.

The diagnosis of *Strobiligera* provided herein addresses the aforementioned issues regarding teleoconch and protoconch

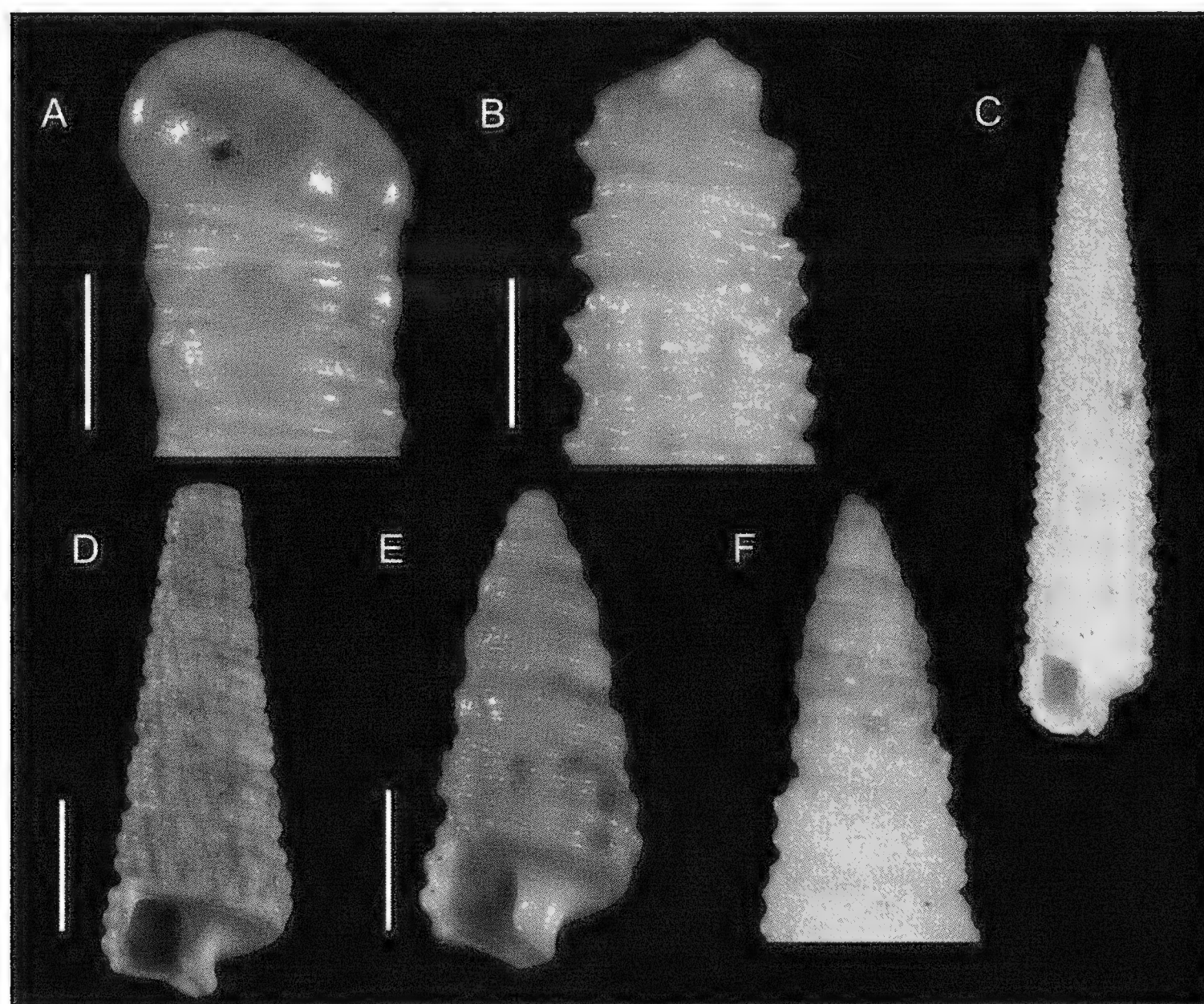**Figure 3. A,** A typical protoconch of *Strobiligera*, like its type species (*S. ibex*); **B,** a typical protoconch of *Inella*, like its type species (*I. gigas*); **C, F;** *Subulophora exporrecta*. **D–E,** *Norephora granulata*. **C, F,** Holotype AMS C.8525. **D,** BGR 1430. **E,** BGR 1432. Scale bars: A–B, E, 500 μm; D, 1mm. (Color shown in electronic version only).

Other genera of the *Inella* group share some similarities with *Strobiligera*. Rolán and Fernández-Garcés (1994) indicated the presence of arrowhead-shaped granules on a species of the genus *Monophorus*; other species within this genus have cruciform granules, just like *Strobiligera*. However, *Monophorus* can be distinguished from *Strobiligera* by the later development of the median spiral cord, whereas in *Strobiligera* the adapical spiral cord is initially reduced.

A very similar genus to *Strobiligera* is *Subulophora* Laseron, 1958, whose Recent species are restricted to the Indo-Pacific, including the type species *Subulophora exporrecta* Laseron, 1958, a junior synonym of *Subulophora rutilans* (Hervier, 1897), according to Marshall (1983). The teleoconch (with an initially reduced adapical spiral cord) and protoconch [embryonic shell with cruciform tubercles, larval shell with a smooth spiral zone above the adapical spiral cord; also, see the description of *S. rutilans* by Marshall (1983)] of *S. exporrecta* (Figs. 3C, 3F) are similar to species of *Strobiligera* with a multispiral protoconch. However, the protoconch of *Subulophora* has an acuminated apex (Fig. 3F), instead of the dome-shaped apex of *Strobiligera* (Figs. 1I, 2I). Furthermore, the adapical spiral cord of the teleoconch of *Subulophora* (Figs. 3C, 3F) develops much faster than that of *Strobiligera* species (Figs. 1A–1E, 2B–2E). Marshall (1983) also stated that *Subulophora rutilans* and *Strobiligera brychia* have similar teleoconchs, but their radulae [that of *S. rutilans* illustrated by Marshall (1983), that of *S. brychia* by Bouchet (1985)] were quite different in number and

relative size of cusps of each tooth, and considered it to be "impossible to ascertain the significance of the differences". We think that their radulae are very different, which increases our confidence in treating them as separate genera.

The fossil genus *Norephora* Gründel, 1975, restricted to Europe, is also similar to *Strobiligera* species with multispiral protoconch. However, the same features used to separate *Subulophora* from *Strobiligera* can be applied to *Norephora*, which raises the assumption of *Norephora* being a synonym of *Subulophora*, pending further investigation. The type material of the type species *Norephora granulata* (Strauch, 1967) is missing (Dr. Michael Amler, pers. comm.), so we illustrate herein the material originally studied by Gründel (1975) when establishing *Norephora* (Figs. 3D–3E).

The number of recognized and named triphorid species in Brazil is currently at 26 (based on Fernandes *et al.* 2013), which includes the two *Strobiligera* species examined herein and excludes the records of *Strobiligera compsa* and *Strobiligera pompona*, as mentioned above. Further studies are needed on the alpha-taxonomy of triphorids from Brazil, including a large revision of the group *Inella s.l.*

ACKNOWLEDGMENTS

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Molecular data reveal an undescribed cryptic species of *Costasiella* Pruvot-Fol, 1951 (Euthyneura: Sacoglossa: Limapontidae) in the Bahamas

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Abstract: Molecular and morphological evidence revealed the existence of two cryptic species of *Costasiella* Pruvot-Fol, 1951 in the Bahamas. A review of the literature indicates that one of these species is the western Atlantic widespread species *Costasiella ocellifera* (Simroth, 1895), whereas the other species is undescribed. The new species is externally similar to *Costasiella ocellifera* but can be distinguished by the absence of a penial stylet and the radular morphology. Phylogenetic and species delimitation analyses confirm that the new species is genetically distinct from *C. ocellifera*. The new species has only been found in the Bahamas, but there are possible records from Cuba and Jamaica.

Key words: cryptic species, mtDNA, nDNA, phylogenetics, species delimitation

Costasiella Pruvot-Fol, 1951 is a small group of sacoglossan sea slugs (Limapontidae Gray, 1847) consisting of 14 valid species (Swennen 2007). *Costasiella* is particularly species rich in the Indo-Pacific tropics and subtropics, with six species described from the Ryukyu Islands alone (Ichikawa 1993), and one from each of the following localities: Japan (Baba 1959, 1961), Micronesia (Marcus 1982), Hong Kong (Jensen 1985), Australia (Jensen 1997), and Thailand (Swennen 2007).

In the Atlantic Ocean, *Costasiella* is represented by a Mediterranean species, *Costasiella virescens* Pruvot-Fol, 1951 and two western Atlantic species, *C. ocellifera* (Simroth, 1895) and *C. nonatoi* Ev. Marcus and Er. Marcus, 1960. A third western Atlantic species described from Brazil, *C. lilianae* (Ev. Marcus and Er. Marcus, 1969) is considered a junior synonym of *C. ocellifera* (see Clark 1984).

In this paper we describe a third western Atlantic species of *Costasiella* collected from the Bahamas. This species has been reported in the literature as a color variation of *C. ocellifera* (see Redfern 2001). However, molecular and morphological data revealed that it is a distinct species.

MATERIAL AND METHODS

Source of specimens

Thirteen specimens initially identified as *Costasiella ocellifera*, two of which have been re-identified as a new cryptic species were sequenced for this study (Table 1). Thirty-two additional specimens were examined morphologically but not sequenced. All specimens were collected in Stocking Island, Exumas, Bahamas. Additional sequences were obtained

from Patrick Krug and GenBank. Specimens were photographed alive, narcotized using a 1M solution of $MgCl_2$, and preserved in 70% EtOH. All the non-type specimens collected are deposited at the Natural History Museum of Los Angeles County (LACM) and the invertebrate collections of the California State Polytechnic University (CPIC). Type material is deposited exclusively at the LACM. Egg masses were obtained from individuals isolated in plastic containers and photographed in the field.

Morphological examination

Two specimens of each species were dissected to remove the pharynx. For each specimen tissue surrounding the radula was dissolved by leaving the pharynx submerged in 10% NaOH overnight. The radulae were rinsed in distilled water, dried, mounted, and sputter coated for examination with a scanning electron microscope (SEM) Hitachi S-3000N at the LACM. Several specimens of *Costasiella ocellifera* and one of the new species were dissected under a Nikon SMZ-1000 dissecting microscope with a camera lucida attachment to investigate the morphology of the reproductive system. The penises of two specimens were removed and photographed with an Olympus CX-31 compound microscope.

DNA extraction

DNA extraction was performed using a hot Chelex® protocol. Approximately 1–3 mg of the foot was cut into fine pieces for extraction. For the Chelex® extraction, the foot tissue was rinsed and rehydrated using 1.0 mL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) for 20 min. A 10% (w/v) Chelex® 100 (100–200 mesh, sodium form, Bio-Rad) solution was prepared using TE buffer. After rehydration, the

Table 1. Material used in the phylogenetic analysis, including locality information, collection date, voucher numbers, and GenBank accession numbers. An asterisk denotes the outgroup.

Species	Locality	Collection date	Voucher number	GenBank Accession Numbers		
				16S	COI	H3
C. ocellifera	Stocking Island, Bahamas	Dec 15, 2007	CPIC 00019	KF411384	-	KF411405
C. ocellifera	Stocking Island, Bahamas	Feb 16, 2009	CPIC 00096	KF411385	KF411397	KF411406
C. ocellifera	Stocking Island, Bahamas	Feb 16, 2009	CPIC 00097	KF411386	-	KF411407
C. ocellifera	Stocking Island, Bahamas	Feb 16, 2009	CPIC 00098	KF411387	KF411398	KF411408
C. ocellifera	Stocking Island, Bahamas	Jan 06, 2009	CPIC 00099	KF411388	-	KF411409
C. ocellifera	Stocking Island, Bahamas	Dec 26, 2009	CPIC 00252	KF411389	KF411399	KF411410
C. ocellifera	Stocking Island, Bahamas	Jan 26, 2010	CPIC 00267	KF411390	KF411400	KF411411
C. ocellifera	Stocking Island, Bahamas	Dec 28, 2009	CPIC 00283	KF411391	-	KF411412
C. ocellifera	Stocking Island, Bahamas	Dec 28, 2009	CPIC 00284	KF411392	KF411401	KF411413
C. ocellifera	Stocking Island, Bahamas	Dec 28, 2008	CPIC 00285	KF411393	KF411402	KF411414
C. ocellifera	Stocking Island, Bahamas	Dec 31, 2008	CPIC 00100	KF411394	KF411403	KF411415
C. ocellifera	St. George, Bermuda	Jun 6, 2006	-	KF438670	KF438672	KF438674
C. ocellifera	Key West, Florida	Oct, 2006	-	KF438669	KF438671	KF438673
C. ocellifera	Bahamas	-	-	DQ480216	DQ471253	DQ534806
C. kuroshimae*	Guam	-	-	DQ480215	DQ471252	DQ534805
C. nonatoi	Bocas del Toro, Panama	2005	-	GU191037	GU191065	-
C. patricki	Stocking Island, Bahamas	Dec 15, 2007	LACM 3241	KF411395	KF411404	KF411416
C. patricki	Stocking Island, Bahamas	Dec 31, 2008	LACM 3242	KF411396	-	KF411417

tissue mixture was then centrifuged, 975.00 mL of the supernatant was removed, and 175.00 mL of the Chelex® solution was added. Samples were then heated in a 56 °C water bath for 20 min, heated in a 100 °C heating block for 8 min, and the supernatant was then used for PCR.

PCR amplification and sequencing

Histone-3 universal primers (H3AF 5′-ATGGCTCG TACCAAGCAGACGGC-3′, H3 AR 5′-ATATCCTTGGG CATGATGGTGAC-3′) developed by Colgan *et al.* (1998), 16S rRNA universal primers (16S ar-L 5′-CGCCTGTTTAT CAAAAACAT-3′, 16S br-H 5′-CCGGTCTGAACTCAGAT CACGT-3′) developed by Palumbi (1996), COI universal primers (LCO1490 5′-GGTCAACAAATCATAAAGATATT GG-3′, HCO2198 5′-TAAACTTCAGGGTGACCAAAAAAT CA-3′) developed by Folmer *et al.* (1994) were used to amplify the regions of interest for most specimens. The opisthobranch-specific COI primers (NAF-COI 5′-GCC TTTTCAACAAACCATAAAGA-3′, NAR-COI 5′-CCATCCT GGTAAAATTAAAATATA-3′) developed by Ornelas-Gatdula *et al.* (2011) were used to amplify the COI fragment in the new species.

The master mix was prepared using 34.75 μL H$_2$O, 5.00 μL Buffer B (ExACTGene, Fisher Scientific), 5.00 μL 25 mM MgCl$_2$, 1.00 μL 40mM dNTPs, 1.00 μL 10mM primer 1, 1.00 μL 10mM primer 2, 0.25 μL 5 mg/mL Taq, and 2.00 μL extracted DNA. The polymerase chain reactions (PCR) were run on an Eppendorf Mastercycler personal thermocycler. Reaction conditions for H3 (universal) and 16S (universal) were as follows: heated lid at 105 °C and initial denaturation of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, followed by a final elongation of 72 °C for 7 min. Reaction conditions for COI (universal) were as follows: heated lid at 105 °C and initial denaturation of 95 °C for 3 min, 35 cycles of 94 °C for 45 s, 45 °C for 45 s, and 72 °C for 2 min, followed by a final elongation step of 72 °C for 10 min. NAF-COI and NAR-COI external primers (used for specimen LACM 3241) had the same reaction as the COI (universal) primers, except for the number of cycles which was changed to 39 cycles.

PCR products yielding bands of appropriate size (approximately 395 bp for H3, 495 bp for 16S, and 750 bp for COI) were purified using the Montage PCR Cleanup Kit (Millipore). Cleaned PCR samples were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Each primer was diluted to 4.0 pmol/μL to send out for sequencing with the PCR products. PCR products were diluted to 6.0, 7.5, and 11.5ng/μL for H3, 16S, and COI respectively. Samples were sequenced at the City of Hope DNA Sequencing Laboratory (Duarte, CA) using chemistry types BigDye V1.1 for fragments less than 500 bp and BigDye V3.1 for fragments larger than 500 bp. One specimen (LACM 3241) was sequenced at the Eton Bioscience, Inc. (San Diego, CA).

Molecular analyses

Sequences for each gene were assembled and edited using Geneious Pro 4.7.4 (Drummond *et al.* 2010). Geneious was also used to extract the consensus sequence between the primer regions, to construct the alignment for each gene using the default parameters, and to concatenate the alignments. Analyses were conducted with trimmed and untrimmed alignments with no changes in the final results. The total length of the aligned sequences ranged between 305–328 bp for H3, 440–454 bp for 16S, and 501–658 bp for COI.

To assess whether H3, 16S, and COI have significantly conflicting signals the incongruence length difference (ILD) test (Mickevich and Farris 1981, Farris *et al.* 1994), implemented in PAUP*4.0 as the partition homogeneity test (Swofford 2002), was conducted for all genes combined.

The levels of saturation for each gene and for the first and second versus third codon positions of COI and H3 were investigated using the substitution saturation test developed by Xia *et al.* (2003) and Xia and Lemey (2009) implemented in the program DAMBE (Xia and Xie 2001). *Costasiella kuroshimae* Ichikawa, 1993 was selected as the outgroup and *Costasiella nonatoi* was included for comparison with *Costasiella ocellifera*. Since *C. nonatoi* and *C. ocellifera* are sympatric it is assumed that they are more closely related than the Japanese species *C. kuroshimae*.

The Akaike information criterion (Akaike 1974) was executed in MrModeltest (Nylander 2004) to determine the best-fit model of evolution. MrModeltest selected the models GTR+G for CO1, GTR+I+G for 16S, and HKY+I for H3 (CO1 γ shape = 0.1446; 16S γ shape = 0.4241, proportion of invariant sites = 0.3832; H3 proportion of invariant sites = 0.8899). Bayesian analyses were executed in MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001), partitioned by gene (unlinked). The Markov chain Monte Carlo analysis was run with two runs of six chains for ten million generations, with sampling every 100 generations. Effective sample sizes and convergence of runs were assessed using Tracer 1.4.1 (Rambaut and Drummond 2007). The default 25% burn-in was applied before constructing majority-rule consensus tree(s).

Diagnostic nucleotides for each clade were identified visually in the alignments after collapsing identical haplotypes using the program Collapse 1.2 (Posada 2004). The Species Delimitation plugin (Masters *et al.* 2011) for Geneious was used to provide a statistical framework to help determine whether clades obtained in the phylogenetic analysis have identity as distinct species. The statistics implemented were the ratio between the mean distance within the members of the clade and the mean distance of those individuals to the nearest clade and the p ID, which represents the mean probability, 95% confidence interval, for a member of the putative species to fit inside (strict p ID), or at least to be the sister group (liberal p ID) of the clade made up by the other individuals belonging to this species (Table 2). Additionally, the Automatic Barcode Gap Discovery (ABGD) method (Puillandre *et al.* 2012) was implemented to compute the theoretical maximal limit of the intraspecific diversity (using a coalescent model) with COI and 16S. ABGD identifies in the entire distribution of pairwise distances which gap (superior to the maximal limit of the intraspecific diversity), potentially corresponds to the so-called 'Barcoding gap,' a hypothetical limit between intra- and interspecific diversity (Puillandre *et al.* 2012). Inference of the limit and gap detection are then recursively applied to previously obtained groups to get finer partitions until there is no further partitioning. The online version of the software (http://wwwabi.snv.jussieu.fr/public/abgd/) was used to analyze the COI and 16S datasets. MEGA 4.0 (Tamura *et al.* 2007) was used to build the distance matrices for COI and 16S using a Tamura Nei model. The data was analyzed using the two available models: Jukes-Cantor (JC69) and Kimura (K80). The program requires two user-specified values: P (prior limit to intraspecific diversity) and X (proxy for minimum gap width). To evaluate the effect on the datasets X values from 0.1 to 5 were tested and the maximum Pmax value was extended from 0.1 to 0.2.

RESULTS

The saturation analysis showed insignificant levels of saturation for all three genes, 16S (Iss = 0.1765 < Iss.c = 0.7062, P = 0.0000), COI (Iss = 0.2620 < Iss.c = 0.7385, P = 0.0000), and H3 (Iss = 0.0341 < Iss.c = 0.6755, P = 0.0000). The ILD test showed non-significant conflicting signals between the genes combined: CO1 vs. H3 (P = 1.00), 16S vs. H3 (P = 0.99), and CO1 vs. 16S (P = 1.00).

The combined analysis of the three genes (H3, 16S, and CO1) produced a consensus Bayesian tree (Fig. 1) in which

Table 2. Species delimitation results from the Bayesian tree.

Species	Closest Species	Monophyly	Intra dist	Inter dist closest	Intra/Inter	P ID(strict)	P ID(liberal)
C. nonatoi	*C. ocellifera*	Yes	0.000	0.612	0.00	0.0	0.96
C. ocellifera	*C. patricki*	Yes	0.018	0.175	0.11	0.94	0.98
C. patricki	*C. ocellifera*	Yes	0.007	0.175	0.04	0.57	0.96
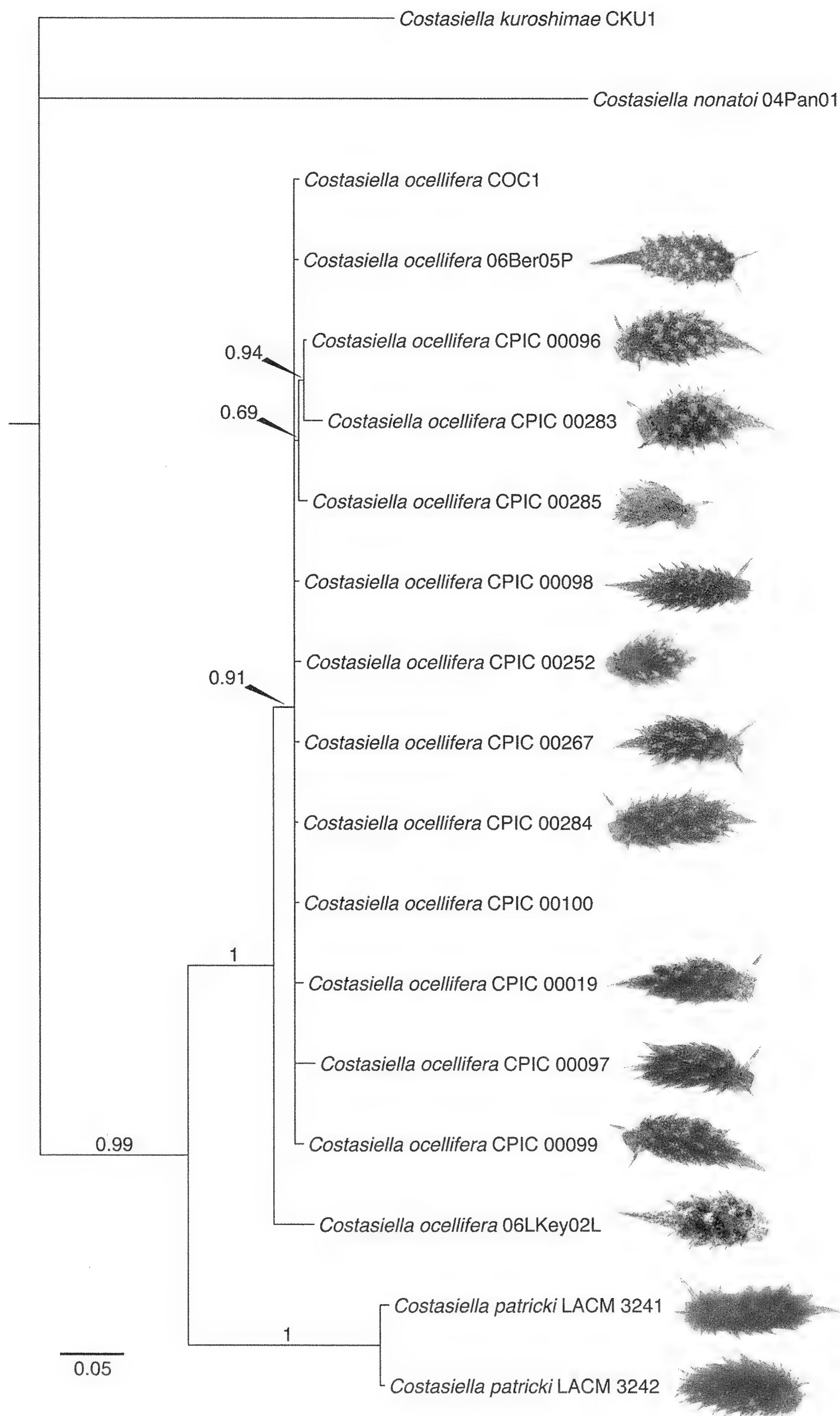
Figure 1. Bayesian consensus tree of the specimens examined with posterior probability values and illustrations of the specimens from which sequences were obtained.

the monophyly of *Costasiella ocellifera* is well supported (pp = 1), including specimens from the Bahamas, Bermuda and the Florida Keys. However, two specimens from the Bahamas form another monophyletic group (pp = 1), sister to *C. ocellifera* (pp = 0.99). These two specimens are herein referred to as *Costasiella patricki*, new species.

The species delimitation analysis (Table 2) for *Costasiella ocellifera* and *C. patricki* shows that values of the ratio between the average distance between the samples of one group (Intra Dist) and the average distance between those samples and the closest clade (Inter Dist) are small, below 0.25. This means that genetic differences within all clades are small relative to the differences between clades, so that there is a well-defined separation of clades. The probability (p ID) of a new sequence fitting inside (strict) or at least as sister group (liberal) of its clade is high in all cases. The results for *Costasiella nonatoi* are not discussed since only one specimen of this species was included and, therefore, meaningful analyses are not possible.

The ABGD analysis with the standard settings recovered 2 groups for COI and 16S respectively. These groups correspond to the clades recovered in the phylogenetic analyses (*Costasiella ocellifera* and *Costasiella patricki*) providing further support for the taxonomic decisions. ABGD grouping results were independent from the chosen model (Jukes-Cantor and Kimura) and unaffected by changes of prior limit of intraspecific variation or X-value.

Because of the genetic differences between specimens of *Costasiella ocellifera* and *Costasiella patricki*, the latter is formally described as a distinct species in systematics descriptions section below and compared morphologically with *C. ocellifera*.

SYSTEMATICS DESCRIPTIONS

Costasiella ocellifera (Simroth, 1895)
(Figs. 2A, 3A, 4A, 5A)
Doto ocellifera Simroth, 1895: Simroth 1895: 168–170, pl. 20, figs. 6–10.
Costasiella ocellifera (Simroth, 1895): Clark 1984: 92–33, fig. 32.
Stiliger lilianae Ev. Marcus and Er. Marcus, 1969: Marcus and Marcus 1969: 7–12, figs. 22–28.
Ercolania lilianae (Ev. Marcus and Er. Marcus, 1969): Marcus 1977: 7.
Costasiella lilianae (Ev. Marcus and Er. Marcus, 1969): Thompson 1977: 137–138, figs. 26j, 32a.

Material examined

Stocking Island, Bahamas (23.5333°N, 75.7667°W), between 1–3 m depth, 18 February 2005, 6 specimens (LACM 172285); 18 February 2005, 6 specimens (LACM 172286); 12 December 2005, 1 specimen (LACM 173222); 17 December 2005, 1 specimen (LACM 173223); 3 February 2007, 5 specimens, dissected (CPIC 00021); 3 February 2007, 5 specimens (CPIC 00022); 15 December 2007, 4 specimens (CPIC 00019) (1 specimen sequenced, isolate EE70), 31 December 2008, 1 specimen (CPIC 00100) (isolate EE88); 6 January 2009, 5 specimens (CPIC 00099) (1 specimen sequenced, isolate EE77); 26 January 2009, 2 specimens (CPIC 00267) (1 specimen sequenced, isolate EE81); 16 February 2009, 1 specimen (CPIC 00096) (isolate EE74); 16 February 2009, 1 specimen (CPIC 00097) (isolate EE75); 16 February 2009, 1 specimen (CPIC 00098) (isolate EE76); 26 December 2009, 3 specimens (CPIC 00252) (1 specimen sequenced, isolate EE80); 28 December 2009, 1 specimen (CPIC 00283) (isolate EE82); 28 December 2009, 1 specimen (CPIC 00284) (isolate EE83); 28 December 2009, 1 specimen (CPIC 00285) (isolate EE84).

External morphology

Background translucent grayish white, with large orange patch on the head, running from the anterior end of the body to the pericardium, between the rhinophores (Figs. 2A). Body covered with dark brown or black spots, varying in density among specimens. Cerata variable in color, from yellow in some juvenile specimens to dark green, covered with numerous opaque white spots of varying sizes in the green animals, and black spots in the yellow animals. Each ceras has a large light blue spot. A sub-apical yellow to orange ring occupies approximately 1/4 to 1/7 of the length of each ceras, followed by a white ring and a black tip. Rhinophores translucent, with numerous dark spots more densely concentrated mid-length, forming a dark band. A bright pale blue spot surrounded by a black ring is located between the eyes and the pericardium.
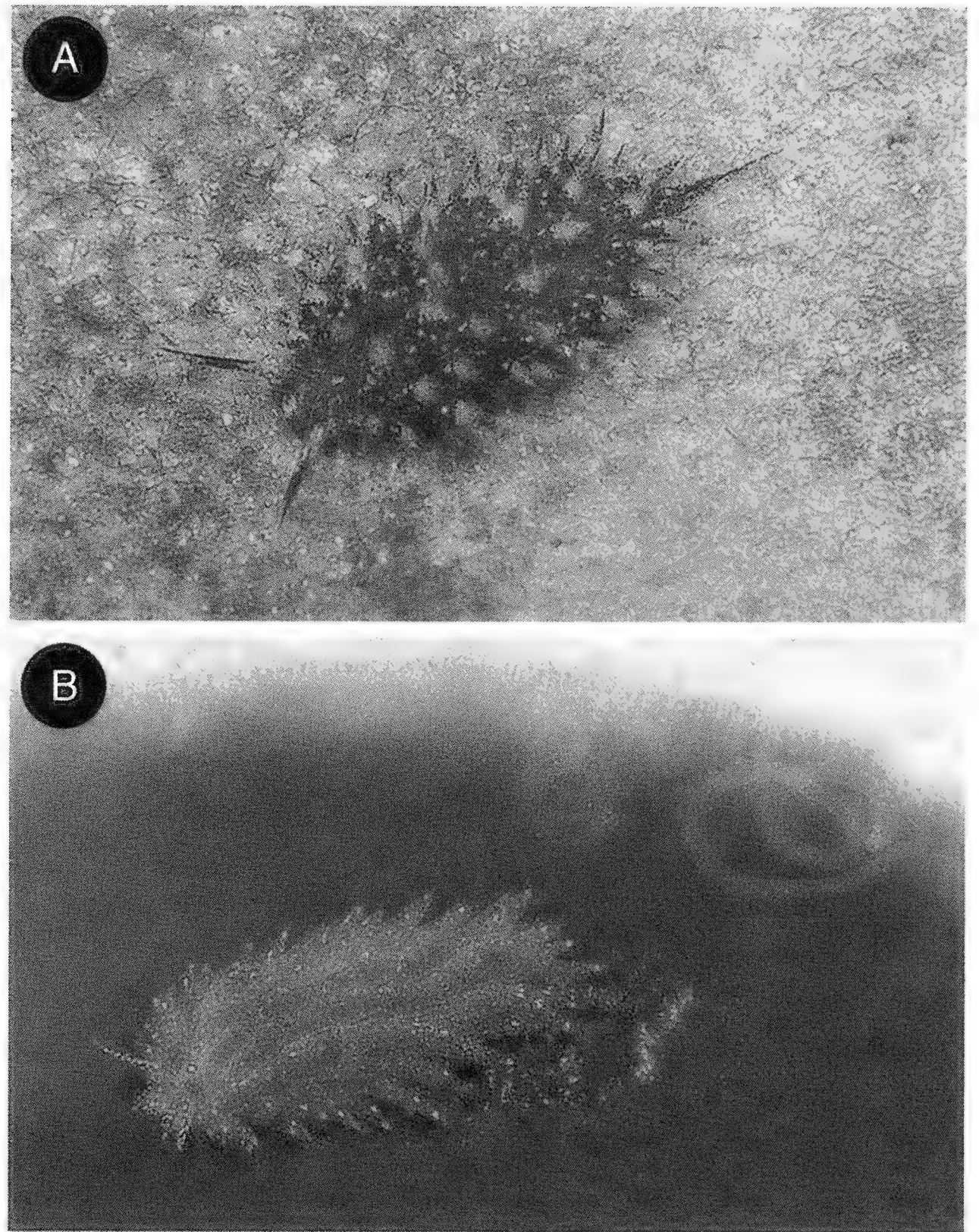
Figure 2. Live animals on *Avrainvillea* photographed in Stocking Island, Bahamas. **A,** *Costasiella ocellifera*, 12 December 2005, and **B,** *Costasiella patricki* n. sp., 18 February 2005, there is no direct evidence the two eggs masses in the photo were deposited by the specimen illustrated.

Body elongate, covered with numerous cerata. Cerata inflated, narrow at the base and wider mid-length, narrowing again apically into a very long and narrow apex. Head almost square, with two inconspicuous and blunt anterior extensions. Rhinophores elongate, smooth, narrow. Anterior end of the foot with two blunt, lateral expansions. Foot sole divided into a longer and wider anterior portion that occupies 2/3 of the length of the body and a shorter and narrower posterior section. Posterior end of the body triangular, lacking ramifications of the digestive gland, distally ending in a narrow tip. Eyes large, close together, situated between the rhinophores. Anus located on the anterior right hand side of the pericardium.

Penial morphology

The penis is elongate connected to a relatively long and convoluted deferent duct, and having a hollow penial stylet on the tip (Figs. 3A, 4A).

Radular morphology

Radular with 23 teeth (CPIC 00099: 7 mm long), 9 in the descending limb and 14 in the ascending limb (Fig. 5A). Teeth
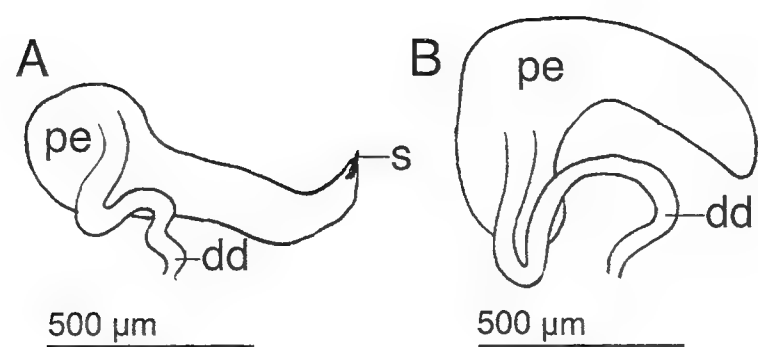
Figure 3. Drawings of the penial morphology. **A,** *Costasiella ocellifera* (CPIC 00021), and **B,** *Costasiella patricki* n. sp. (CPIC 00020). Abbreviations: **dd,** deferent duct; **pe,** penis; **s,** penial stylet.

elongate, with sharp tips, and lacking denticles. Teeth base short, concave at the base, followed by a narrowing of the tooth, a widening, another narrowing and a widening into the apical end.

Biology

Costasiella ocellifera feeds on the green alga *Avrainvillea longicaulis* (see Cimino and Ghiselin 2009) and *A. nigricans* (see Redfern 2001). It contains a brominated diphenylmethane derivative, avrainvilleol that has been shown experimentally to deter feeding by fish (Hay *et al.* 1990). This species displays poecilogony, with intracapsular metamorphosis and planktonic larvae. The developmental mode varies at the population level and appears to be fixed over the lifetime of an individual (Ellingson 2006). In the Bahamas we observed egg masses with similar egg sizes (Fig. 6A–C) suggesting only one developmental mode is found in this population. The egg mass is a ribbon of eggs forming a spiral with 1.5–2.5 whorls (Fig. 6A–C). The eggs are arranged in transverse rows of 4–6 eggs. Some egg masses are more tightly packed and coiled, and have more whorls (Fig. 6A, C) than others (Fig. 6B).
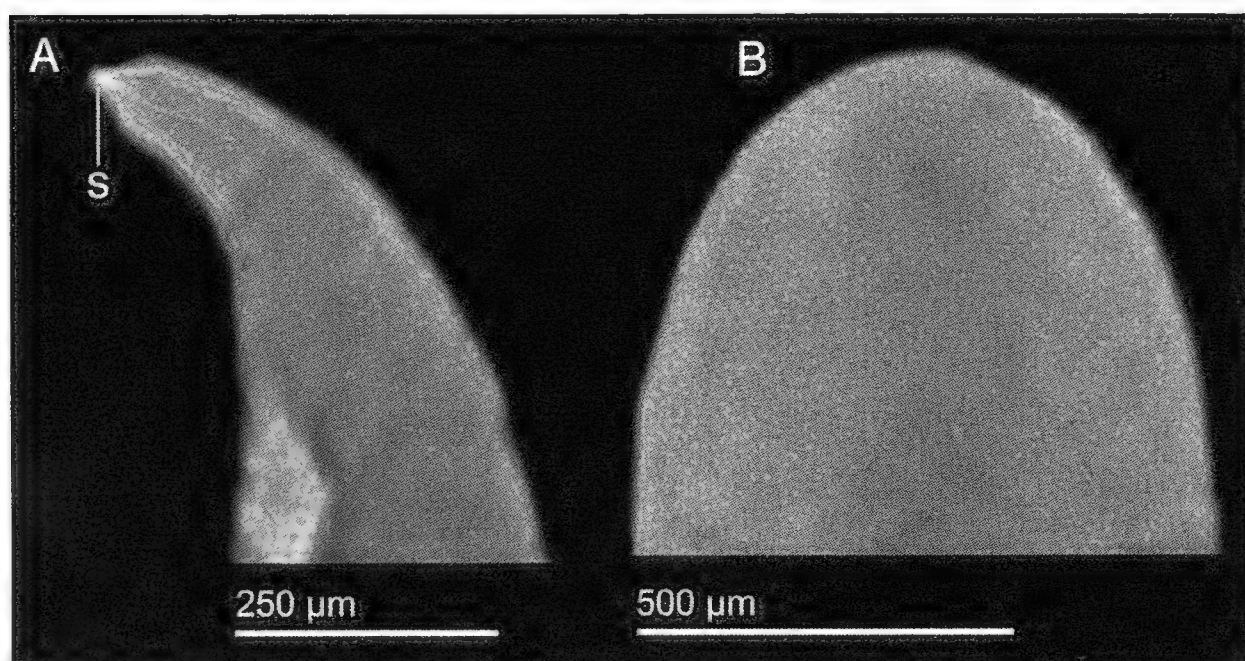
Figure 4. Photographs of the distal end of the penis under a compound microscope. Because of pressure from the coverslip the penises are artificially deformed. **A,** *Costasiella ocellifera* (CPIC 00021), and **B,** *Costasiella patricki* n. sp. (CPIC 00020). Abbreviation: **s,** penial stylet.
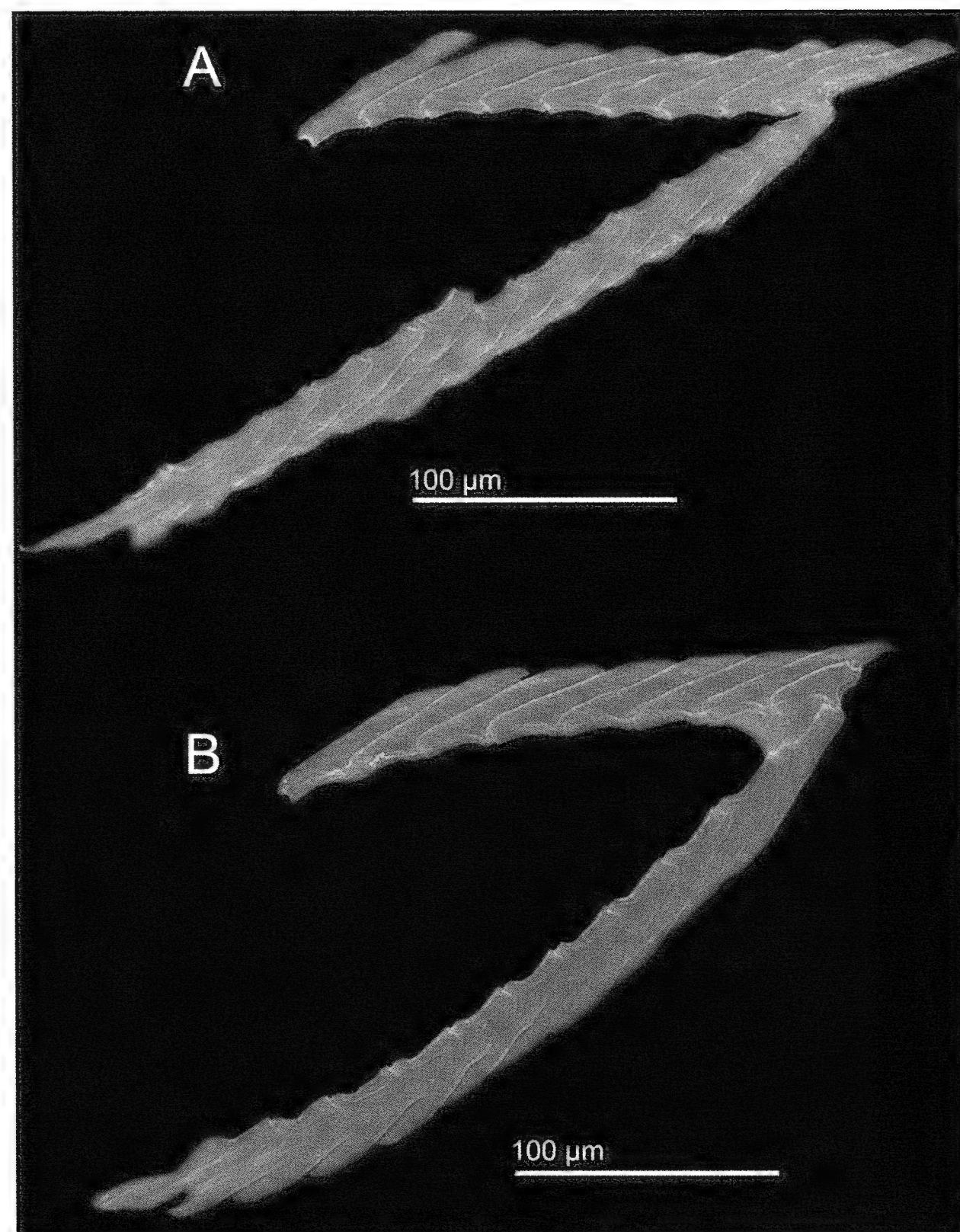
Figure 5. Scanning electron micrographs of the radulae. **A,** *Costasiella ocellifera* from Bahamas (CPIC 00099), and **B,** *Costasiella patricki* n. sp., paratype (LACM 3242).

Costasiella patricki new species

(Figs. 2B, 3B, 4B, 5B)

Costasiella ocellifera (Simroth, 1895): Redfern 2001: fig 681B.

Type material

Holotype: Stocking Island, Bahamas (23.5333°N, 75.7667°W), 2 m depth, 15 December 2007, 8 mm long, preserved in 70% ethanol (LACM 3241) (isolate EE90).

Paratype: Stocking Island, Bahamas (23.5333°N, 75.7667°W), 2 m depth, 31 December 2008, 1 specimen 5 mm long, pharynx dissected, preserved in 70% ethanol (LACM 3242) (isolate EE91).

Additional specimens

Stocking Island, Bahamas (23.5333°N, 75.7667°W), 3 February 2007, 4 specimens, destroyed for dissection (CPIC 00020).

External morphology

Background orange, covered with numerous black spots and some opaque white patches (Fig. 2B). Cerata pale to dark

Figure 6. Photographs of egg masses. **A,** *Costasiella ocellifera*, 18 February 2005, **B,** *C. ocellifera*, 18 February 2005, **C,** *C. ocellifera*, 18 February 2005, and **D,** *C. patricki* n. sp., 18 February 2005.

Penial morphology

The penis is wide, connected to a relatively long and simple deferent duct and devoid of armature (Figs. 3B, 4B).

Radular morphology

Radular with 19 teeth (LACM 3242: 5 mm long), 8 in the descending limb and 11 in the ascending limb (Fig. 5B). Teeth elongate, with sharp tips, and lacking denticles. Teeth base wide, concave at the base. Teeth blade with a constant width except for the apex, which narrows into a sharp, thin tip.

Biology

Costasiella patricki n. sp. is found on the green algae *Avrainvillea* spp. often together with *Costasiella ocellifera*. Only one egg mass of this species was observed (Fig. 6D). The egg mass is a ribbon of eggs forming a spiral with 4.5 whorls (Fig. 6D). The eggs are arranged in transverse rows of 6–7 eggs. Although the eggs were not measured with precision tools, they appear to be small suggesting this species could have planktonic larvae.

Etymology

This species is named after our colleague Patrick Krug, who has worked extensively on the systematics and ecology of Caribbean sacoglossans. He also provided nuclear and mitochondrial DNA sequences of two specimens of *Costasiella ocellifera* that were essential for the molecular analysis of this study.

DISCUSSION

Costasiella ocellifera and *Costasiella patricki* n. sp. are genetically and morphologically distinct and, therefore, are treated as different species. The presence of unique diagnostic characters in each species as well as the results of the species delimitation analysis confirmed their distinctiveness. However, the external morphological differences only became obvious when sequence data became available. The two species are very similar and often difficult to distinguish. Both *C. ocellifera* and *C. patricki* n. sp. often have a greenish appearance (because of the green cerata), although some juvenile

green, covered with black dots, each bearing a large light blue spot. A sub-apical yellow to orange ring occupies approximately 1/8 to 1/10 of the length of each ceras, surrounded by a translucent white area proximally, and white tip with black dots distally. Rhinophores translucent, with numerous dark spots and an orange area mid-length. A bright pale blue spot surrounded by a black ring is located between the eyes and the pericardium.

Body elongate, covered with numerous cerata. Cerata inflated, narrow at the base and wider mid-length, narrowing again apically into a long and narrow apex. Head with two blunt anterior extensions clearly visible. Rhinophores elongate, smooth, narrow. Anterior end of the foot with two blunt, lateral expansions. Foot sole divided into a longer and wider anterior portion that occupies 2/3 of the length of the body and a shorter and narrower posterior section. Posterior end of the body triangular, lacking ramifications of the digestive gland, distally ending in a narrow tip. Eyes large, close together, situated between the rhinophores. Anus located on the anterior right hand side of the pericardium.

Figure 7. Reproductions of published illustrations of western Atlantic *Costasiella*. **A,** Original description of *Doto ocellifera* (from Simroth 1895), **B,** Redescription of *Costasiella ocellifera* (from Clark 1984), **C,** Original description of *Stiliger lilianae* (from Marcus and Marcus 1969), and **D,** Redescription of *Costasiella lilianae* (from Thompson 1977).

specimens of *C. ocellifera* may appear yellowish. Also, both species have a conspicuous blue spot behind the rhinophores and anterior of the pericardium. The main external difference between the two species is that *C. patricki* n. sp. is often lighter in color and the orange rings on the cerata are smaller, less conspicuous.

The internal morphological differences between the two species are more obvious, and include the absence of a penial stylet in *Costasiella patricki* n. sp., which is present in *Costasiella ocellifera*. The specimens dissected were very small, thus it was impossible to reliably describe other internal organs with gross anatomical examination.

Costasiella ocellifera and *Costasiella patricki* n. sp. also have different egg masses, having as many as 4 whorls in *C. patricki* n. sp. and only 1.5–2.5 whorls in *C. ocellifera*. However, because egg mass morphology, egg size and development is variable in *C. ocellifera*, this is probably not a reliable trait to distinguish the two species.

A review of the literature described below revealed that there is no available name for *Costasiella patricki* n. sp. and, therefore, is here described as a new species. The taxonomy of western Atlantic *Costasiella* has been problematic with two species names introduced for *C. ocellifera* and controversies regarding the number of valid species as well as their generic

allocation. The first comprehensive description of a western Atlantic *Costasiella* was by Marcus and Marcus (1969), who described *Stiliger lilianae* from southern Brazil including detailed illustrations of the preserved animals (Fig. 7C). Baba and Hamatani (1970) suggested that this species should be transferred to *Costasiella*, but Marcus (1976) disagreed and included it in *Ercolania* Trinchese, 1872, based on the rhinophoral morphology. *Stiliger lilianae* was reported from Puerto Rico (Marcus 1976), Florida (Marcus 1977), and Jamaica (Thompson 1977). Thompson (1977) agreed with Baba and Hamatani (1970) including *Stiliger lilianae* in *Costasiella* because of the location of the eyes, close together between the rhinophores (Fig. 7D). The original description of *Stiliger lilianae* (Marcus and Marcus 1969) is very complete. It includes detailed anatomical descriptions and drawings of the reproductive system and the radula, and a reference to the presence of a penial stylet, later confirmed by Marcus (1982). This is consistent with the characteristics of the specimens here assigned to *C. ocellifera*. Clark (1984) studied animals collected from near St. George's Harbor, Bermuda, the type locality of *Doto ocellifera*, and concluded that this species is in fact a member of *Costasiella* and a senior synonym of *Stiliger lilianae*. The original description of *D. ocellifera* (Fig. 7A) shows the distinctive eye structure of this species as well as other external characteristics summarized by Clark (1984). The specimens illustrated by Clark (1984) (see Fig. 7B) also match the original description of *Stiliger lilianae* and the characteristics of the animals here assigned to *C. ocellifera*.

More recently, Miles and Clark (2002) found different egg masses produced by specimens of *Costasiella ocellifera* from two different populations in the Florida Keys. These two populations have different developmental modes (planktotrophic and intracapsular metamorphosis). However, Miles and Clark (2002) could not find any morphological differences between members of the two populations. They proposed two hypothesis to explain their observations: poecilogony and the existence of two cryptic species. In a more comprehensive study, Ellingson (2006) showed that a single poecilogonous species was involved. *Costasiella patricki* n. sp. does not correspond to any of these populations in the Florida Keys. Patrick Krug (pers. comm.) has extensively

sampled populations of *C. ocellifera* in Florida and other Caribbean locations with different developmental modes, but has never collected or sequenced *C. patricki* n. sp. Other published records and illustrations of *C. ocellifera* from the Dominican Republic (Domínguez 2003), the Bahamas (Poddubetskaia 2004), the US Virgin Islands (Wilk 2005), Belize and Jamaica (Valdés *et al.* 2006), and Barbados (Alleyne 2006), clearly belong to *C. ocellifera*. The only unquestionable record of *C. patricki* n. sp. available in the literature is from the Bahamas. Redfern (2001, figs. 800A–D) illustrated the color variation of *C. ocellifera* in the Bahamas, and whereas fig. 681A corresponds to *C. ocellifera*, fig. 681B corresponds to *C. patricki* n. sp. In a second edition of this work, Redfern (2013) included additional photos showing the variability of *C. ocellifera*, but in this case none of them belong to *C. patricki*. Other published photos by Espinosa *et al.* (2006, fig. 415) from Cuba and Gundersen (2003) from Jamaica, could correspond to *C. patricki*, as they represent specimens with light green cerata bearing small orange rings and numerous black dots. However, this should be confirmed with molecular and morphological data.

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Description of four new species of *Ledella* Verrill and Bush, 1897 (Pelecypoda: Nuculanidae) off the coast of Brazil, using a morphometric approach

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Abstract: As a result of deep-water oil prospecting in the Campos Basin, Brazil, a rich diversity of protobranchiate pelecypods has been revealed. In this study, we identified four new species of *Ledella* and conducted a morphometric study to corroborate their delimitation. The resulting conchological-based statistic models are in 95% agreement with the traditional criteria for the right valves and 88.33% for the left valves. Certain variables were particularly relevant in these analyses; these variables expressed the importance of the shape of the rostrum and hinge plate for the right valves and the general shell shape and hinge plate for the left valves. The hinge plate variables are not typically used as diagnostic characters of protobranchiate pelecypods and, when applied, they are not given much importance. However, our statistical analyses stressed the importance of these characters. Therefore, we strongly suggest that future descriptions of protobranchiate pelecypods place greater emphasis on these characters.

Key words: Biodiversity, deep sea, hinge plate, morphometry, Bivalvia.

As a consequence of oil prospecting in the Campos Basin (20–23°S) by the oil company Petrobras, deep-water samples have been obtained, which have increased our knowledge of deep-sea mollusks from the southeast of Brazil over the last 10 years (*e.g.,* Domaneschi and Lopes 1990, Absalão *et al.* 2003, Caetano *et al.* 2006, Zelaya *et al.* 2006, Simone and Cunha 2008, Oliveira and Absalão 2009, Passos and Birman 2009, Figueira and Absalão 2010).

Protobranchia is the most abundant group of Pelecypoda in the deep-sea (400–5000 m), which constitutes the largest habitat on Earth, with at least 94% of the seabed lying below the permanent thermocline (Allen and Sanders 1996a). Recently, Benaim and Absalão (2011a, b, c) increased the number of protobranchiate Pelecypoda known in Brazilian waters by approximately 27%.

Ledella Verrill and Bush, 1897 is a genus that has a complex taxonomic history, including changes of the type species (Warén 1978, 1981, ICZN 1985, Allen and Hanna 1989). Information about this group in Brazilian waters is currently lacking. The most recent Brazilian catalogue of mollusks (Rios 2009) listed only *Ledella solidula* (Smith, 1885)and *Ledella semen* (Smith, 1885), with the latter given under the generic name of *Nuculana* Link, 1807. This does not reflect the present knowledge of this genus in Brazil. Five species of *Ledella* are currently known to be present in Brazilian waters: *L.semen* and *L. solidula,* as reported by Smith (1885); *Ledella ultima* (Smith, 1885) and *Ledella orixa* (Dall, 1927), as reported by Allen and Hanna (1989); and *Ledella acinula* (Dall, 1889), as reported by Allen and Sanders (1996b).

Recently, Sharma *et al.* (2013) completed a major molecular analysis of the Protobranchia, showing that the genus *Ledella* is not monophyletic. However, for the use of species delimitation, we have analyzed all the species here described as a unit.

Like the majority of protobranchiate Pelecypoda, *Ledella* has a conservative shell shape, subtle interspecific differences and a lack of clear boundaries between genera. The species within this genus form groups or clusters with conchological similarity, as occurs in *Yoldiella* Verrill and Bush, 1897(La Perna 2004, Benaim and Absalão 2011a). The species presented in this paper are no exception to this rule, and due to their similar shell outline, a morphometric analysis was required to corroborate their delimitation into different species. Therefore, this paper contributes to the knowledge of the genus *Ledella* from Brazil by describing four previously unknown species together with their morphometric data.

MATERIAL AND METHODS

The samples used in the study were collected during three projects. First, the "Live Resources of the Economic Exclusive Zone" (Revizee) project, which took place during 2001 (Table S1) and was sponsored by the Brazilian government as part of international demands to retain Brazilian authority over the 200 miles of territorial sea. This project extended across the Brazilian continental margin and the methodological details are given in Amaral and Rossi-Wongtschowski (2004) and Lavrado and Ignacio (2006). Second, the "Environmental

Characterization of Campos Basin, RJ, Brazil" (Oceanprof) project, which was conducted during the years 2002 and 2003 (Table S1). Finally, "Habitats Project – Campos Basin Environmental Heterogeneity", which was developed during 2008 and 2009 (Table S2). The two latter projects were sponsored directly by Petrobras and were carried out using the research vessel *Astro–Garoupa* equipped with a box corer. Details about the associated methodological procedures can be found in Lavrado and Brasil (2010). All biological material was sorted under magnification and the best-preserved specimens of each species were examined and photographed under a scanning electron microscope (ZEISS EVO 40) at the Gerência de Bioestratigrafia e Paleoecologia Aplicada (BPA) of the Petrobras Research Center (Centro de Pesquisas da Petrobras – CENPES). Live-collected specimens were very common in the material obtained from the Habitats Project, which constituted most of the examined material. All of the shells from the Revizee and Oceanprof projects were empty. The specimens studied have been deposited primarily in the mollusk collection of the Instituto de Biologia, Universidade Federal do Rio de Janeiro (IBUFRJ), and secondarily at the Museu Nacional do Rio de Janeiro (MNRJ), the Museu Oceanográfico Eliézer de Carvalho Rios, Universidade Federal do Rio Grande (MOFURG), and the Muséum national d'Histoire naturelle (MNHN), Paris.

All of the *Ledella* samples studied here were first designated as morphospecies. We conducted an exploratory analysis based on the Population Aggregation Analysis (Davis and Nixon 1992) using 1362 valves and taking into account the "traditional" criteria used in bivalve taxonomy (Cox 1969, Bailey 2009, Killeen and Turner 2009), which are based on conchological characters. The identifications were made through comparisons with figures of the type species and descriptions available in the literature (Smith 1885, Laghi 1984, Allen and Hanna 1989, Laghi and Palazzi 1989, La Perna 2004, Rios 2009, Allen and Sanders 1996b).

Morphological orientation in pelecypods is usually related to a series of axes (Bailey 2009). To determine dorsal and ventral height (DH and VH, respectively), we used the antero-posterior orientation based on an imaginary line passing under the adductor muscles scars, which divides the body into dorsal and ventral areas (Allen 1985).

Measurements

Considering the inequivalve condition of these species, the valves were separated to obtain measurements of both left and right valves. Whenever possible, the valves measured came from the same individual. This was not always feasible because the measurements of the hinge teeth require that they are well-preserved.

The valves were photographed, together with a scale, using a Canon Power Shot G10 camera attached to a Zeiss Stemi SV11
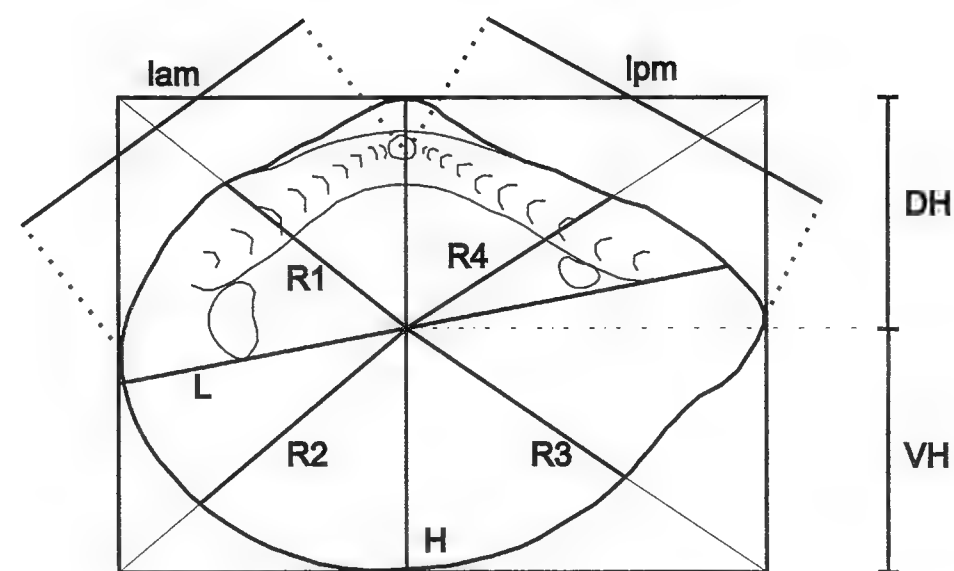
Figure 1. Measurement scheme for the general shell shape. Total length and height (L and H, respectively); dorsal and ventral height (DH and VH, respectively); length of the antero and postero-dorsal margin (lam and lpm, respectively); and lengths of the four lines beginning near the shell center and radiating toward the four corners of the rectangle delimiting the shell (R1, R2, R3, and R4).

stereomicroscope. The photographs were then analyzed to obtain the measurements, using the software Axio Vision release 4.6.3 (4.2007). To ensure the valves were parallel to the plane of the camera lens, a platform made of playdough was used.

Measurements representing the general shell shape were taken as follows: total length (L); total height (H); dorsal height (DH); ventral height (VH); length of the antero-dorsal margin (lam); length of the postero-dorsal margin (lpm); and lengths of the four lines beginning near the shell center and radiating toward the four corners of the rectangle delimiting the shell (R1, R2, R3, and R4) (Fig.1). Similar approaches were used by Knudsen (1970), Rabarts and Whybrow (1979), Warén (1989), Rhind and Allen (1992), Allen *et al.* (1995), Bonfitto and Sabelli (1995), Fuiman *et al.* (1999), Benaim and Absalão (2011b), and Benaim *et al.* (2011).

The measurements taken for the hinge plate were as follows: length of the anterior and posterior parts of the hinge plate (ahp and php, respectively); width of the anterior part of the hinge plate and its teeth (wap and wat, respectively); and width of the posterior part of the hinge plate and its teeth (wpp and wpt, respectively), as previously conducted by Benaim and Absalão (2011b). Furthermore, ten additional measurements were taken with the valves in dorsal view, replicating the method of Benaim *et al.* (2011): width of the valve (W); total length of the hinge plate (lhp); dorsal length of the anterior and posterior parts of the hinge plate (dahp and dphp, respectively); height of the highest tooth of the anterior and posterior parts of the hinge plate (hat and hpt, respectively); and lengths between the center of the highest tooth of the anterior and posterior parts of the hinge plate to the umbo (alu and plu, respectively) and to the respective margins of the valve (alm and plm, respectively) (Fig.2). These hinge plate measurements taken in the dorsal view have been used only once previously, by Benaim *et al.* (2011) on the genus *Yoldiella*.
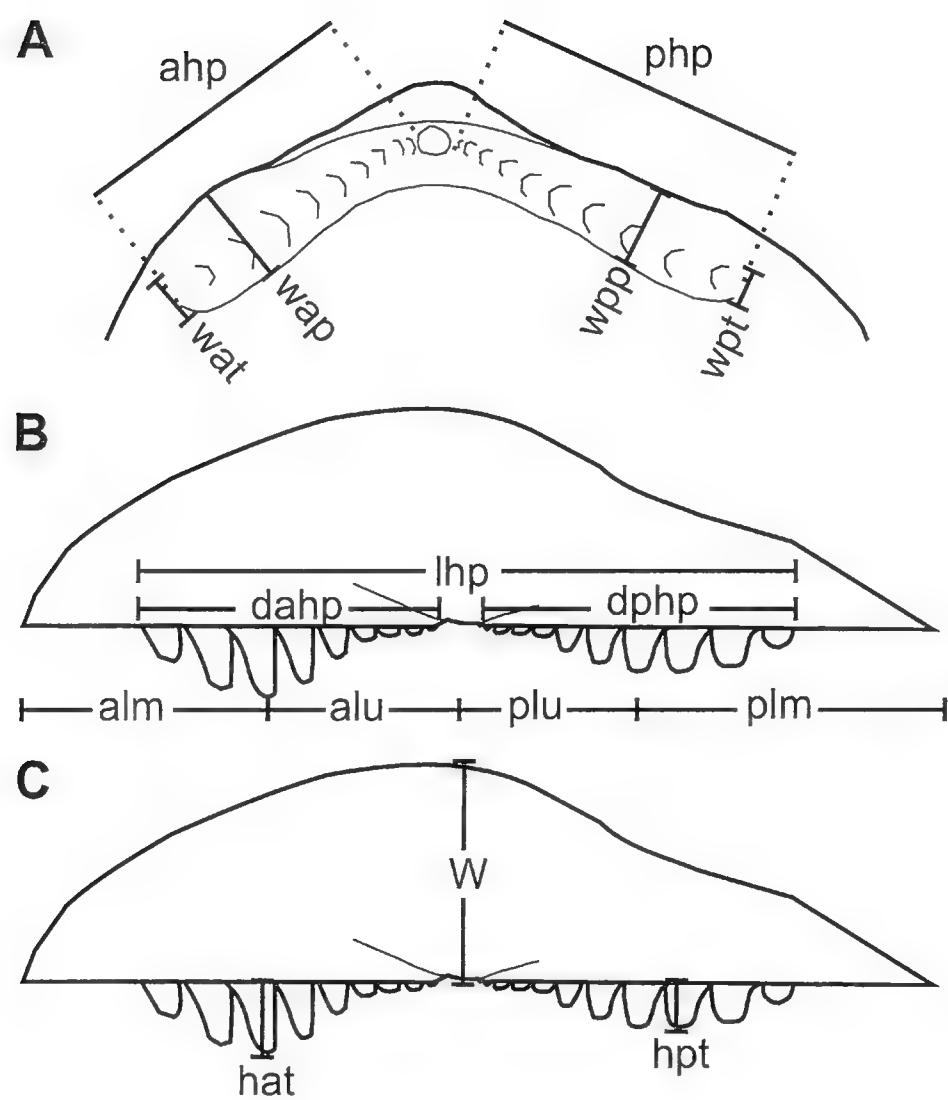
Figure 2. Measurement scheme for the hinge plate and width of the valve. **A,** length of the anterior and posterior parts of the hinge plate (ahp and php, respectively); width of anterior part of the hinge plate and teeth (wap and wat, respectively); and width of the posterior part of the hinge plate and teeth (wpp and wpt, respectively). **B,** total length of the hinge plate (lhp); dorsal length of the anterior and posterior parts of the hinge plate (dahp and dphp, respectively); and lengths between the center of the highest tooth of the anterior and posterior parts of the hinge plate to the umbo (alu and plu, respectively) and to the respective margins of the valve (alm and plm, respectively). **C,** width of the valve (W); and height of the highest tooth of the anterior and posterior parts of the hinge plate (hat and hpt, respectively).

Figure 3. Measurement scheme for the shape of the rostrum. Height of the rostrum (Hr); length of the rostrum (Lr); dorsal and ventral heights of the rostrum (Hrd and Hrv, respectively); and profundity of the rostrum's sinuosity (Prs).

An additional set of five measurements was implemented in this study: height of the rostrum (Hr), measured by a line that borders the first tooth of the posterior hinge plate and goes straight from the dorsal to the ventral margins of the valve; length of the rostrum (Lr), measured perpendicularly from the Hr line to the edge of the rostrum; dorsal and ventral heights of the rostrum (Hrd and Hrv, respectively), using the Lr line to divide the dorsal and ventral regions of the rostrum; and profundity of the rostrum's sinuosity (Prs), which is the greatest linear distance from the lower point of the sinuosity to the imaginary line of continuity of the valve (Fig. 3).

We also calculated 22 ratios: ahp/lam, alm/plm, alu/alm, dahp/dphp, H/L, hat/dahp, hat/hpt, hat/W, hpt/dphp, hpt/W, Hr/Lr, Hr/H, Hrd/Hrv, lhp/L, Lr/L, Lr/plm, php/lpm, plu/plm, W/L, W/lhp, wap/ahp and wpp/php.

Morphometric analyses

One could argue that the above ratios will inevitably be strongly correlated with the variables that were utilized in the calculation of these ratios, leading to redundancy in the

analysis. To avoid this problem, in addition to redundancy between the variables themselves, we performed a preliminary correlation analysis among all variables and excluded one of each pair of values with strong correlation ($r \geq 0.9$). The normality of variables was verified through normal probability plots and, when necessary, appropriate transformations were implemented to normalize the data.

Discriminant function analysis was used to determine if these morphospecies could be objectively identified through the defined variables and, if so, which of the analyzed features were the most important for their discrimination. Forward stepwise procedures were employed to select the most useful discriminating variables. To perform these analyses, we standardized the morphometric data as described by Romesburg (1984). All statistical procedures were performed using STATISTICA software (version 7.0), StatSoft, Inc., Tulsa, Oklahoma.

The analyses were conducted for two separate subgroups: the right valves and the left valves. Taking into account the inequivalve condition of these species, a joint analysis would not have provided information about which group of valves could determine the morphospecies more accurately nor about the features of each valve which are the most important for morphospecies discrimination.

Taxonomic Discussion

Each morphospecies considered in this study was first compared with the co-generic species previously reported in Brazilian waters. They were then compared with the co-generic species that have similar shell shapes and, finally, with the other morphospecies described in this work.

Only Recent species were used to discuss the new species described herein. Thus, somewhat similar species such as *Ledella peraffinis* (Seguenza, 1877) (Miocene), whose neotype was figured by Laghi and Palazzi (1989), *Ledella pusio* (Philippi, 1844) (Pliocene), which was reviewed and figured by Laghi (1984), and *Ledella seminulum* (Seguenza, 1877) (Pleistocene-Pliocene) and *Ledella nicotrae* (Seguenza, 1877) (Pliocene), both figured by La Perna *et al.* (2004), were not considered in the discussion. Besides the chronological distance between the Recent and fossil species, all of these fossil species are from the Mediterranean Sea.

RESULTS

A traditional taxonomic descriptive approach suggested the existence of four morphospecies which provided the base for the morphometric procedures.

Right Valves

For this analysis, only nine variables were excluded due to strong correlation with the other variables. The excluded variables were: L, H, DH, lam, php, R2, R3, R4 and lhp. The remaining 44 variables were included in the analysis and 25 of them were retained in the final model, which was in 95% agreement with the traditional criteria (Table 1).

This rate of agreement was achieved using three discriminant functions (df). The first one (df1) represents the role of the shape of the Rostrum (Prs, Lr and Lr/plm) in the discrimination. Df2 represents the joint role of the Rostrum Height (Hrd e Hrd/Hrv) and Hinge Plate Width (wap and wpp) in the discrimination. Df3 represents the role of the Hinge Plate (hpt, wap/ahp, wap and hpt/W) in the discrimination. These three roles were also perceived during the traditional and more intuitive taxonomic discrimination of the individuals.

Three variables (ahp/lam, H/L and wpt) made a relatively small contribution to the model, but we decided to retain them because excluding them from the analysis generated a model with only 14 variables and 86.66% agreement with the traditional criteria, which is 8.34% lower than the first result.

Left Valves

For this analysis, only nine variables were excluded due to strong correlation with the others. These variables were: L, H, DH, lam, php, R3, R4, lhp and hpt. The remaining 44 variables were included in the analysis and 24 of these were retained in the final model, which showed 88.33% agreements with the traditional criteria (Table 2), 6.67% less than the result obtained for the right valves.

This rate of agreement was achieved using three discriminant functions. The first one (df1) represents the roles of the width of the valve (W) and of the distance of the highest

Table 1. Standardized coefficients for canonical variables for the right valves analysis. The most relevant variables in each axis are marked in dark gray; the second most representative variables in each axis are marked in light gray. The variables which made minor contributions are marked in italics. Df, discriminant function.

	Df1	Df2	Df3
Prs	0.84685	0.57349	0.08940
W	0.40454	0.14923	1.79748
ahp/lam	-0.34177	*0.51987*	*0.54561*
lhp/L	0.75184	-0.27905	0.44486
Plu	-0.48379	0.59059	0.82643
Alm	0.59928	-0.29880	2.74110
Hr/Lr	-0.98651	-1.26410	-0.70248
Ahp	0.20366	0.22406	-2.66803
H/L	*0.36787*	*-0.48827*	*-0.05886*
hat/dahp	0.62724	0.05129	0.82733
Alu	0.26413	-0.56459	0.05034
Hat	-1.01679	0.29528	-0.27112
Lpm	0.74758	-0.52086	-0.45604
Hrd	0.34414	2.25031	0.70813
alm/plm	-0.59847	0.09829	-2.33328
Hrd/Hrv	-0.70831	-1.84689	-0.70744
Lr	-1.73270	-1.34534	-2.74996
Hpt	-1.16240	0.20600	-3.62005
Wpt	*0.44496*	*-0.28454*	*0.51637*
Wpp	-0.36244	0.81991	-0.11739
Wat	-0.02904	0.70197	-0.09151
wap/ahp	-0.35423	0.75593	-4.37649
Lr/plm	1.04844	-0.61189	2.15145
Wap	0.54872	-1.48020	4.63718
hpt/W	0.61434	-0.61345	2.37309
Eigenval	3.70612	1.51830	1.05303
Cum.Prop	0.59039	0.83225	1.00000

tooth of the anterior part of the hinge plate to its respective margin (alm); df2 represents the role of the expansion of the antero-ventral region of the valve (R2) in the discrimination; df3 represents the role of the ratio between the size of the highest tooth of the posterior part of the hinge plate and the width of the valve (hpt/W) in the discrimination. In addition, in all three functions the ratio between the width of the valve and its length (W/L) was one of the two main variables: the ratio between the dorsal length of the hinge plate and the length of the valve (lhp/L) was one of the main variables in df1 and one of the two main variables in df2 and df3, and the ratio between the width of the valve and the dorsal length of the hinge plate (W/lhp) was one of the two main variables in df2 and df3. The presence of these variables in more than one discriminant function reinforces the importance of the width of the valve, the length of the hinge plate and the length of the valve to the discrimination of the morphospecies. The prominence of these variables in the

Figure 4. Canonical analysis scatterplots for the right valves, combining all three discriminant functions (df).

Table 2. Standardized coefficients for canonical variables for the left valves analysis. The most relevant variables in each axis are marked in dark gray; the second most relevant variables in each axis are marked in light gray. The variables that made minor contributions are marked in italics. Df, discriminant function.

	Df1	Df2	Df3
Hr/Lr	1.216731	0.33400	-0.42661
W	-0.807000	-1.69353	0.66830
H/L	-0.732078	0.54540	-1.49951
alm	1.332459	0.10327	-0.65290
php/lpm	*-0.274477*	*0.00366*	*0.09067*
ahp/lam	*0.060819*	*-0.33352*	*-0.18710*
lhp/L	-0.801864	-7.11341	-7.70510
Lpm	-0.098203	1.17700	-0.46356
wpt	-0.226754	0.42273	0.91259
ahp	0.661153	1.17329	1.74951
R1	-0.193882	-1.79420	0.91022
Prs	*-0.292743*	*-0.40283*	*0.10688*
hat/hpt	*0.286672*	*0.15125*	*-0.35170*
W/L	1.921255	10.84032	13.32499
W/lhp	-0.658799	-6.81776	-6.09872
alu	-0.755089	1.14047	1.01010
R2	0.829580	1.d64855	-1.60351
VH	-0.474442	-1.70245	2.36178
hpt/W	-0.125905	0.60649	4.35272
hpt/dphp	0.102861	-0.25046	-3.73286
alu/alm	1.292262	-1.03185	-1.65989
dphp	0.125179	-0.81548	-4.69959
wpp	*-0.150277*	*-0.42347*	*-0.34195*
wat	*0.430124*	*0.29482*	*-0.07997*
Eigenval	2.318400	1.96972	0.66221
Cum.Prop	0.468332	0.86623	1.00000

in which the shape of the rostrum was important to the discrimination whilst the width and length of the valve were only minor contributors.

Six variables (php/lpm, ahp/lam, Prs, hat/hpt, php and dahp) made relatively small contributions to the model, but we again decided to retain these, in accordance with the first analysis. Removing these variables from the analysis generated a model with 18 variables and with 82.5% of the cases in agreement with the traditional criteria, which is 5.83% lower than the first result.

Hinge Plate

We also decided to test the discriminatory power using the hinge plate features alone, following Benaim *et al.* (2011). The analyses were again performed using the right and left valves in separate subgroups, and in both cases, nine variables were included in the model. For the right and left valves respectively, the models showed 66.66% and 64.16% agreement with the traditional criteria (Table 3).

three discriminant functions shows the importance of the hinge plate and the width and length of the valve in this analysis, in contrast to the results of the right valve analysis,
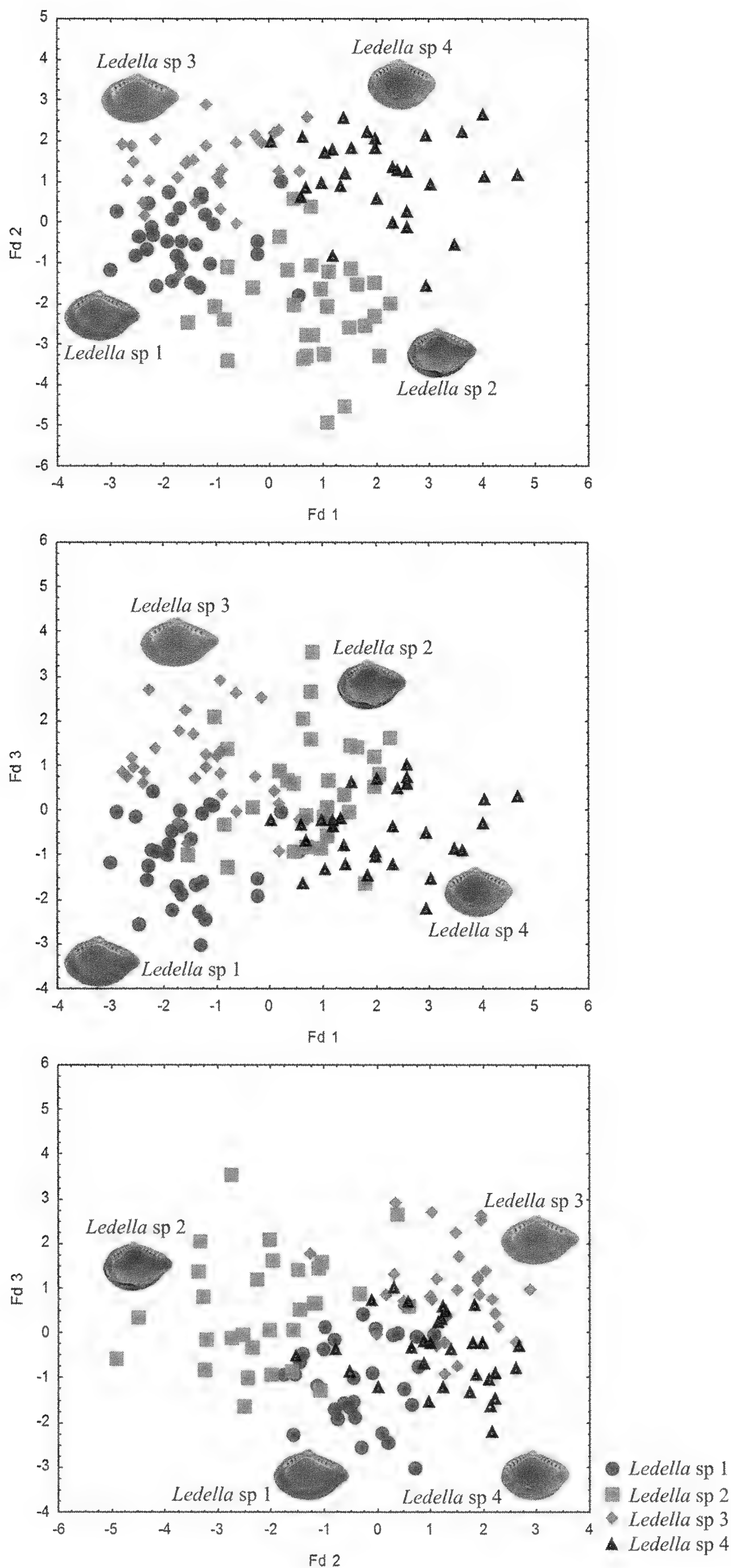
Figure 5. Canonical analysis scatterplots for the left valves, combining all three discriminant functions (df).

These rates of success were achieved using three discriminant functions (df) for each analysis. For the right valves analysis, the first one (df1) represents the tridimensional shape of the anterior part of the hinge plate; df2 represents the thickness of the posterior part of the hinge plate; df3 represents the relative positions of the highest teeth from anterior and posterior parts of the hinge plate. For the left valves, df1 represents the relative positions of the highest teeth from anterior and posterior parts of the hinge plate; df2 represents the shape of the posterior part of the hinge plate; df3 represents the relative position of the highest tooth of the anterior part of the hinge plate on the anterior part of the valve.

Only two variables were important in both hinge plate analyses: alm/plm, which indicates the position of the highest teeth of the anterior and posterior parts of the hinge plate in relation to the margin, and wpp, which is the width of the posterior part of the hinge plate. The first variable may be influenced by the antero-dorsal rostrum present in some of the morphospecies; however, this was not measured. The second variable had been perceived as an important character previously, during the initial discrimination of these morphospecies.

Considering the morphological differences previously detected during the initial analysis of the material and the high support provided by the morphometric approach for the distinction of our initial morphospecies, we describe the four species herein.

Systematics

Order NUCULANIDA Carter, Campbell and Campbell, 2000

Family NUCULANIDAE

Ledella spocki sp. nov.

(Figs. 4 and 5 as *Ledella* sp. 1 and Fig. 6 as *Ledella spocki* sp. nov.)

Holotype

MNRJ 30480, Habitats 7 #F6 R2, 08-VII-2008. 22°19′11″S; 40°05′44″W; 403m.

Paratypes

IBUFRJ 19238, Habitats 7 #H6 R2, 07-VII-2008. 21°44′21″S; 40°05′18″W; 402 m.

MOFURG 51797, Habitats 8 #C6 R3, 31-I-2009. 22°59′00″S; 40°48′28″W; 376.6 m.

MNHN IM-2010-21015, Habitats 7 #B6 R2, 04-VII-2008. 23°12′31″S; 40°58′31″W; 447.4m.

Material examined (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0201/suppl_file/Viegas_2014_Suppl_docs.pdf)

Etymology

This species is named after the fictional character "Spock" from the Star Trek TV and movie series. The overall shape of its valves resembles the shape of the pointed ear of the Vulcans, a humanoid extraterrestrial species depicted in

Table 3. Standardized coefficients for canonical variables for the hinge plate analyses. The most relevant variables are marked in gray. Df, discriminant function.

Right Valves

	df1	df2	df3
Hat	-0.717286	-0.292718	-0.74520
alm/plm	-0.080687	-0.894922	-1.46122
wap/ahp	0.843338	0.364217	-0.98901
plu/plm	-0.476898	0.052819	1.15000
alu/alm	0.199852	-0.538310	-1.10171
wpp	-0.020109	1.016999	-0.13674
wpp/php	-0.474712	-0.401955	-0.05389
wpt	0.034626	-0.873887	0.55696
wat	-0.443634	0.447644	-0.00058
Eigenval	0.848731	0.477082	0.11853
Cum.Prop	0.587624	0.917935	1.00000

Left Valves

	df1	df2	df3
dphp	-1.01472	0.921452	0.37551
alm/plm	-1.51834	0.427152	0.84545
ahp	-1.04440	0.109696	-0.40674
alu/alm	-0.61262	0.470459	0.95693
plu	0.79939	-0.198373	-0.41296
wpp	1.58973	0.734929	0.80325
wpt	-0.36763	-0.690460	-1.17862
wpp/php	-0.85653	-0.017427	0.02813
hat/dahp	-0.36750	0.072022	0.24557
Eigenval	0.98125	0.626145	0.09994
Cum.Prop	0.57473	0.941466	1.00000

this series. Because "Mr. Spock" is the most famous Vulcan in the series, the species has been named after him.

Distribution

Only known from the Campos Basin, off the coast of Rio de Janeiro, Brazil.

Diagnosis

Thin shell with marked anterior and posterior shoulders. Acute rostrum. Profound rostral sinuosity.

Description

Shell oblong–elliptical, inflated, thin, inequivalve, inequilateral, H/L about 0.72; umbones prominent, opisthogyrous and inwardly directed. Antero-dorsal margin convex; anterior margin showing a shoulder. Rounded ventral margin forming a sinuosity leading to an acute rostrate posterior end which points downward; postero-dorsal margin almost straight, with a shoulder near the end of the hinge plate. Smooth outer surface. Hinge plate interrupted by a small and almost rounded resilifer. Posterior part of the hinge plate longer and thinner than anterior one in adult specimens, and almost equal in juveniles. Width of the anterior and posterior rows of teeth occupying about 60% and 58%, respectively, of the total width of the hinge plate of the right valves and about 59% and 55% of the anterior and posterior hinge plates, respectively, of the left valve. Shell microsculpture: the prodissoconch presents a weak granulation and the hinge teeth present pustules on the side that faces the umbones. Maximum shell length: 1.94 mm. Prodissoconch height: 70µm. Prodissoconch length: 73µm. (Table 4)

Remarks

Ledella spocki sp. nov. is similar to *L. semen* with respect to the general shell shape and in the acuteness of its rostrum, but they can be distinguished on the basis of the less opisthogyrous umbones and the more profound rostral sinuosity of *L. spocki* sp. nov.

Ledella spocki sp. nov. shares a long and acute rostrum and a profound rostral sinuosity with *L. solidula*, but *L. spocki* sp. nov. can be distinguished from the latter on the basis of having no concentric lines and a rostrum that is shorter and less downward-pointing than that of *L. solidula*.

Ledella spocki sp. nov. differs from *L. ultima* in that the former has no concentric lines and presents thinner teeth on the hinge plate and a more acute rostrum than *L. ultima*.

Ledella spocki sp. nov. differs from *L. acinula* in that the former has no concentric lines and presents a more acute rostrum and a less inflated posterior region of the shell than *L. acinula*.

Ledella spocki sp. nov. differs from *L. orixa* in that the former presents less opisthogyrous umbones, a larger posterior region in comparison to the anterior region and a smaller anterior part of the hinge plate when compared to the posterior part.

Ledella spocki sp. nov. can be distinguished from *L. acuminata* (Jeffreys, 1870) in that the former has no concentric lines, a shorter hinge plate and presents thinner hinge teeth in relation to the hinge plate thickness, compared with *L. acuminata*.

Ledella legionaria sp. nov.

(Figs. 4 and 5 as *Ledella* sp. 2 and Fig. 7 as *Ledella legionaria* sp. nov.)

Holotype

MNRJ 30481, Habitats 7 #F6 R2, 08-VII-2008. 22°19′11″S; 40°05′44″W; 403m.

Paratypes

IBUFRJ19300, Habitats 9 #H6 R2, 05-II-2009. 21°44′21″S; 40°04′53″W; 404 m.
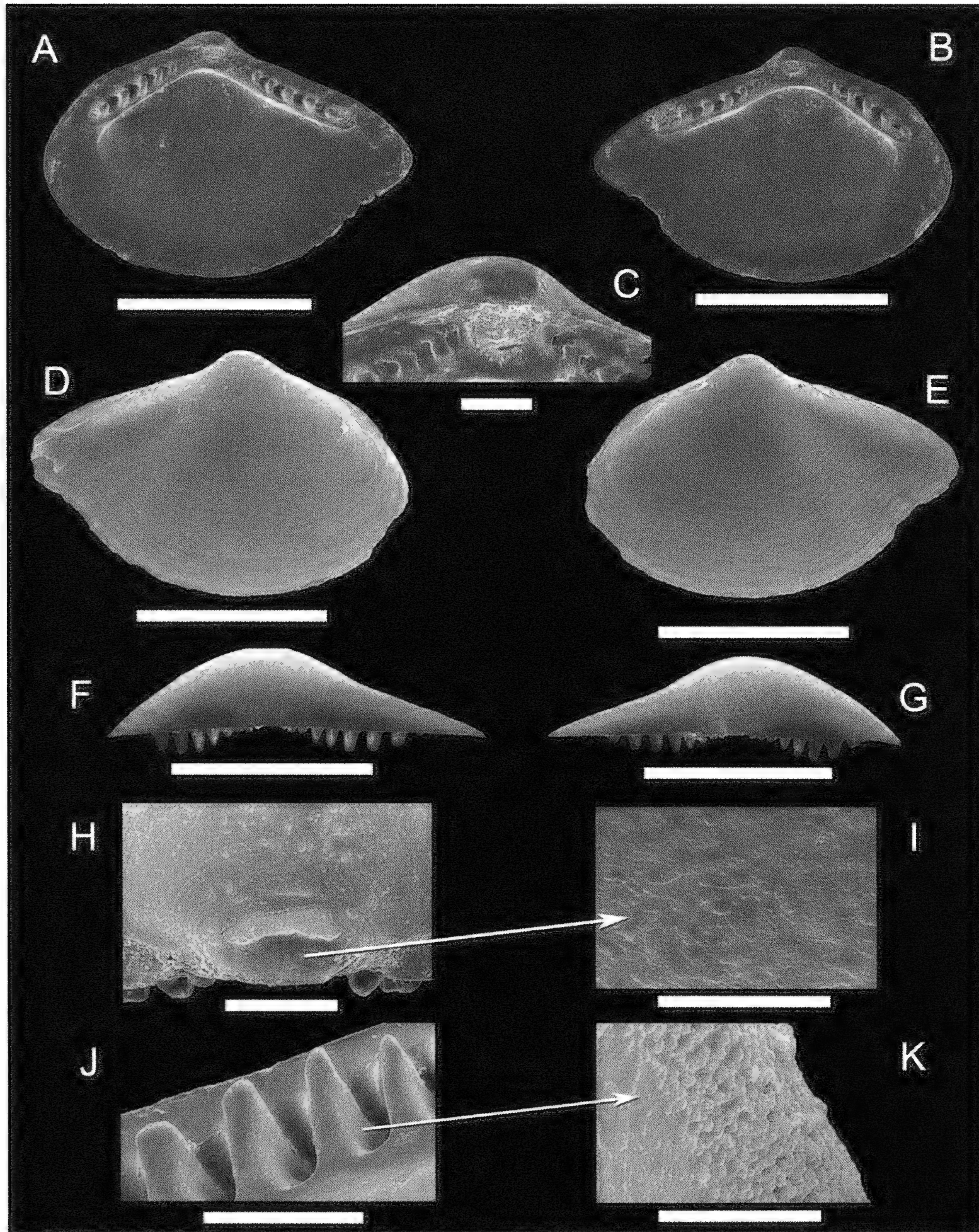
Figure 6. *Ledella spocki* sp. nov. (Holotype MNRJ 30480). Internal view: **A,** right valve; **B,** left valve. External view: **D,** right valve; **E,** left valve. Dorsal view: **F,** right valve; **G,** left valve. Details: **C,** resilifer of the right valve. Prodissoconch (IBUFRJ 19239): **H,** overview; **I,** detail of the tip of the prodissoconch showing some granulation. Hinge teeth (IBUFRJ 19247): **J,** overview; **K,** detail of the tooth showing the tooth pustules. Scales A, B, D–G: 1mm. C, H: 0.1mm. I: 0.01 mm. J: 0.2 mm. K: 0.02 mm.

MOFURG 51798, Habitats 7 #F6 R1, 08-VII-2008. 22°19′10″S; 40°05′42″W; 402.3m.

MNHN IM-2010-21016, Revizee V #41, 20-VII-2001. 21°13′S; 40°13′W; 1000m.

Material examined (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0201/suppl_file/Viegas_2014_Suppl_docs.pdf)

Etymology

This species is named after the Roman legionnaires. The shape of its rostrum resembles the outline of a shoulder armor, similar to those used by the legionaries in their *lorica*

segmentata (segmented plates) armor. Additionally, its shell is thicker than those of the other species described herein, as though the animal wears a heavier armor than the others.

Distribution

Only known from the Campos Basin, Rio de Janeiro, Brazil.

Diagnosis

Shell thick with prominent anterior and posterior shoulders. Rostrum acutely rounded. Profound rostral sinuosity, more profound in the left valve than in the right one.

Description

Shell oblong–elliptical, inflated, thick, inequivalve, inequilateral, H/L about 0.74; umbones prominent, opisthogyrous and inwardly directed. Antero-dorsal margin convex, acute shoulder delimiting antero-dorsal and anterior margins, merging to a convex ventral margin. Ventral margin forming a sinuosity leading to a rostrate posterior end which points slightly downward; postero-dorsal margin almost straight, with a marked shoulder near the end of the hinge plate. Hinge plate interrupted by a deep, rounded resilifer. Posterior part of the hinge plate longer and thinner than anterior one in adult specimens, and almost equal in juveniles. Width of the anterior and posterior rows of teeth occupying about 61% and 56%, respectively, of the total width of the hinge plate on the right valves and about 61% and 57% to the anterior and posterior parts of the hinge plates, respectively, on the left valve. Shell microsculpture: the prodissoconch presents a weak granulation and the hinge teeth present pustules on the side that faces the umbones. Maximum shell length: 2.31 mm. Prodissoconch height: 77 μm, length: 86 μm. (Table 4)

Remarks

Ledella legionaria sp. nov. differs from *L. semen* in having less opisthogyrous umbones, a more profound rostral sinuosity and a less acute rostrum than *L. semen*.

Ledella legionaria sp. nov. differs from *L. solidula* in having no concentric lines and in presenting a prominent shoulder in the antero-dorsal region and a rostrum that is shorter and less acute than that of *L. solidula*.

Ledella legionaria sp. nov. differs from *L. ultima* in having no concentric lines and in presenting thinner teeth on the hinge plate and a more acute rostrum than *L. ultima*.

Ledella legionaria sp. nov. differs from *L. acinula* in having no concentric lines and in presenting a more acute rostrum and a less inflated posterior region of the shell than *L. acinula*.

Ledella legionaria sp. nov. differs from *L. orixa* in presenting less opisthogyrous umbones, a larger posterior region than the anterior region and a smaller anterior part of the hinge plate in comparison to the posterior part.

Compared to *Ledella sublevis* Verrill and Bush, 1898, *L. legionaria* sp. nov. has a similar shell shape, but a less smooth postero-dorsal margin and a longer posterior part of the hinge plate when compared to the anterior part, with thinner hinge teeth than *L. sublevis*.

Compared to *Ledella jamesi* Allen and Hanna, 1989, *L. legionaria* sp. nov. has a similar shell shape, with a shoulder on the postero-dorsal margin, but *L. legionaria* sp. nov. has a longer rostrum and no concentric lines.

Ledella lusitanensis Allen and Hanna, 1989, also has a similar shell shape when compared to *L. legionaria* sp. nov., but the latter has a shoulder in the postero-dorsal margin and a more inequilateral hinge plate, with the posterior part of the hinge plate longer and with thinner teeth than the anterior one.

Compared to *Ledella spocki* sp. nov., *L. legionaria* sp. nov. is larger, thicker, more inflated and has a less profound rostrum sinuosity on the right valve. *Ledella legionaria* sp. nov. also has a shorter rostrum and a more prominent posterior shoulder. *Ledella legionaria* sp. nov. has longer posterior parts of the hinge plate on both valves, but longer anterior parts of the hinge plate are present only on the right valve. These species do not vary much from each other with respect to the thickness of the hinge plate or that of the hinge teeth. Regarding the prodissoconch, they do not differ much in height and microsculpture, but the prodissoconch of *L. legionaria* sp. nov. is longer in length than the prodissoconch of *L.spocki* sp. nov.

Ledella elfica sp. nov.

(Figs. 4 and 5 as *Ledella* sp. 3 and Fig. 8 as *Ledella elfica* sp. nov.)

Holotype

MNRJ 30482, Habitats 7 #H6 R2, 07-VII-2008. 21°44′21″S; 40°05′18″W; 402m.

Paratypes

IBUFRJ 19371, Habitats 7 #H6 R1, 06-VII-2008. 21°42′02″S; 40°06′15′W; 404.7 m.

MOFURG 51799, Revizee Central V #52, 21-VII-2001. 21°46′S; 40°05′W; 450 m.

MNHN IM-2010-21017, Habitats 7 #F6 R2, 08-VII-2008. 22°19′11″S; 40°05′44″W; 403m.

Material examined (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0201/suppl_file/Viegas_2014_Suppl_docs.pdf)

Etymology

This species is similar in shape to the ear of an elf from Norse mythology, so we decided to name this species after those elves.

Distribution

Only known from the Campos Basin, Rio de Janeiro, Brazil.

Diagnosis

Thin shell with anterior and posterior shoulders usually absent; when present, shoulders not prominent. Acute rostrum. Profound rostral sinuosity.

Description

Shell oblong–elliptical, inflated, thin, inequivalve, inequilateral, H/L about 0.72; umbones prominent, opisthogyrous and inwardly directed. Antero-dorsal margin convex; anterior margin rounded, smoothly merging to the slightly convex ventral margin. Ventral margin forming a sinuosity leading to a rostrate posterior end which points downward; postero-dorsal margin almost straight, sometimes weakly sloping downward near the end of the hinge plate. Smooth surface. Hinge plate interrupted by a deep, rectangular resilifer. Posterior hinge plate longer and thinner than anterior one in adult specimens, and almost equal in juveniles. Width of the anterior and posterior rows of teeth occupying about 60% and 59%, respectively, of the total width of the hinge plate of the right valves and about 62% and 59% to the anterior and posterior hinge plates, respectively, of the left valve. Shell microsculpture: the prodissoconch presents a weak granulation and the hinge teeth present pustules on the side that faces the umbones. Maximum shell length: 1.86 mm. Prodissoconch height: 83 μm. Prodissoconch length: 104 μm. (Table 4)

Remarks

Compared to *Ledella semen*, *L. elfica* sp. nov. has a similar shell shape, with a smooth outline and an acute rostrum, but *L. elfica* sp. nov. has less opisthogyrous umbones and a more profound rostral sinuosity.

Ledella elfica sp. nov. is similar to *L. solidula* in that both have an acute rostrum and a profound rostral sinuosity, but they can be distinguished because *L. elfica* sp. nov. does not have concentric lines and its rostrum is shorter and points less downwards than that of *L. solidula*.

Ledella elfica sp. nov. differs from *L. ultima* in having no concentric lines and in presenting thinner teeth on the hinge plate and a more acute rostrum than *L. ultima*.
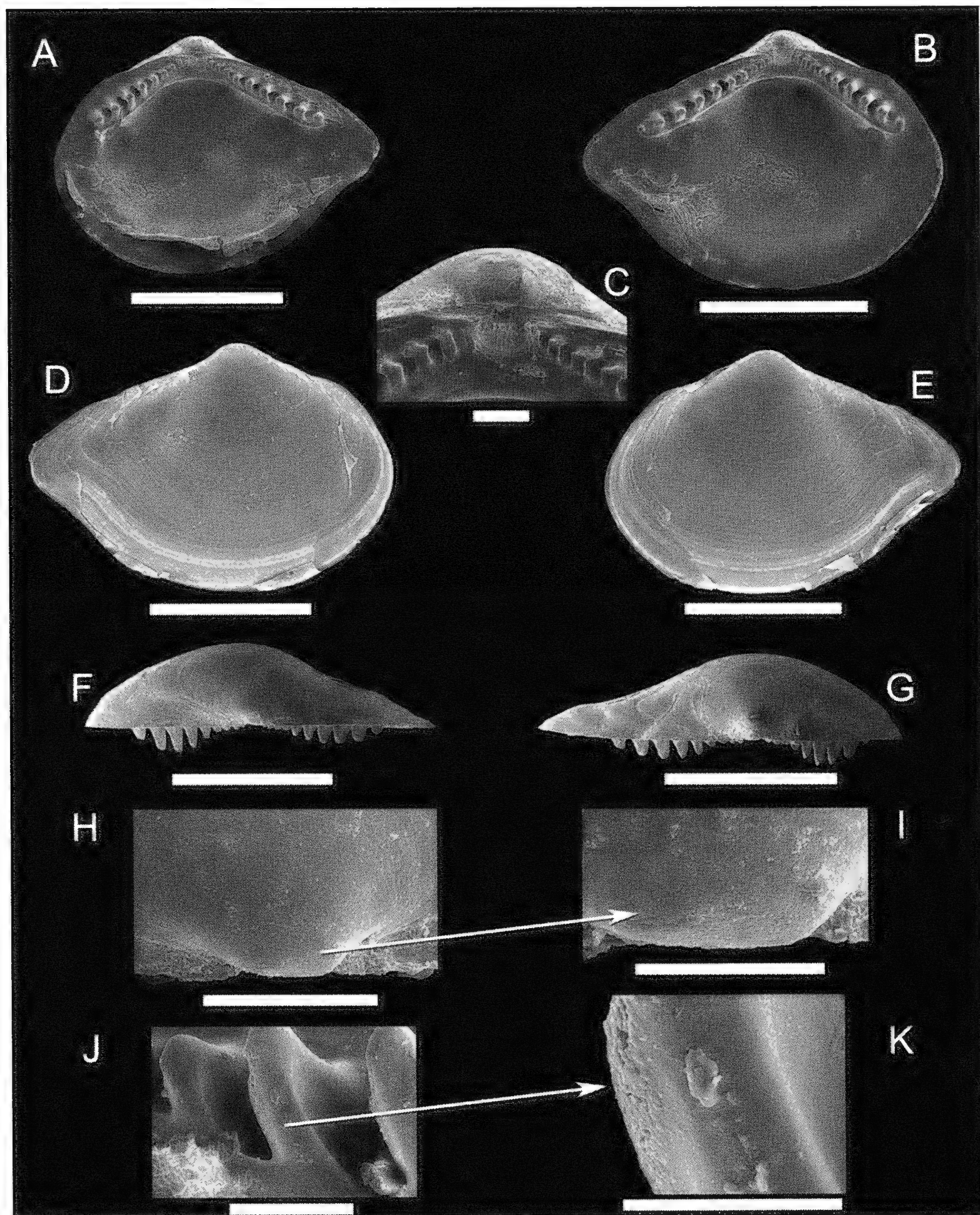
Figure 7. *Ledella legionaria* sp. nov. (Holotype MNRJ 30481). Internal view: **A,** right valve; **B,** left valve. External view: **D,** right valve; **E,** left valve. Dorsal view: **F,** right valve; **G,** left valve. Details: **C,** resilifer of the right valve. Prodissoconch (IBUFRJ 19284): **H,** overview; **I,** detail of the tip of the prodissoconch showing some granulation. Hinge teeth (IBUFRJ 14637): **J,** overview; **K,** detail of the tooth showing the tooth pustules. Scales A, B, D–G: 1 mm. C, H, J: 0.1 mm. I: 0.02 mm. K: 0.05 mm.

no concentric lines, a shorter hinge plate and the hinge teeth occupy less of the hinge plate's width in comparison to *L. acuminata*.

Ledella elfica sp. nov. is similar to *L. spocki* sp. nov. in shape and thickness of the shell and in the length of the rostrum, but they can be distinguished because *L. elfica* sp. nov. is smaller in size (H, L and W), the anterior part of its hinge plate is thinner and longer on both valves and it has a less high and less acute rostrum with a less profound rostral sinuosity than *L. spocki* sp. nov. *Ledella elfica* sp. nov. also has a hinge plate (measured in dorsal view) that is longer in the right valve and shorter on the left one, compared to *L. spocki* sp. nov. Besides that, the hinge teeth of *L. elfica* sp. nov. are longer, in both valves, than those of *L. spocki* sp. nov. Regarding the prodissoconch, they do not differ much in height and microsculpture, but the prodissoconch of *L. elfica* sp. nov. is larger in length than the prodissoconch of *L. spocki* sp. nov.

Ledella elfica sp. nov. is similar to *L. legionaria* sp. nov. with respect to the general shell shape, but they can be distinguished because *L. elfica* sp. nov. has a longer and more acute rostrum than *L. legionaria* sp. nov. and its rostral sinuosity is more profound on the right valve and less profound on the left valve compared to *L. legionaria* sp. nov. With regard to the hinge plates, the hinge teeth of *L. elfica* sp. nov. occupy a larger part of the hinge plate thickness on the posterior part of the hinge plate than that of *L. legionaria* sp. nov. Regarding the prodissoconch, they do not differ much in height and microsculpture, but the prodissoconch of *L. elfica* sp. nov. is larger in length than the prodissoconch of *L. legionaria* sp. nov.

Ledella manati sp. nov.

(Figs. 4 and 5 as *Ledella* sp. 4 and Fig. 9 as *Ledella manati* sp. nov.)

Holotype

MNRJ 30483, Revizee V #504, 02-VII-2001. 14°28′S; 38°54′W; 278m.

Paratypes

IBUFRJ 19398, Habitats 7 #C6 R1, 04-VII-2008. 22°59′00″S; 40°48′25″W; 387.4 m.

MOFURG 51800, Revizee Central V #1, 03-VII-2001. 13°04′S; 38°21′W; 450 m.

MNHN IM-2010-21018, Habitats 7 #F6 R2, 08-VII-2008. 22°19′11″S; 40°05′44″W; 403m.

Ledella elfica sp. nov. differs from *L. acinula* in having no concentric lines and in presenting a more acute rostrum and a less inflated posterior region of the shell than *L. acinula*.

Ledella elfica sp. nov. differs from *L. orixa* in presenting less opisthogyrous umbones, a larger posterior region than the anterior region and a smaller anterior part of the hinge plate when compared to the posterior part.

Ledella elfica sp. nov. is similar to *L. acuminata* in the general outline of the shell, with an acute rostrum, a profound rostral sinuosity and the presence of a smooth shoulder on the postero-dorsal margin, which is found in some specimens of *L. elfica* sp. nov. However, *L. elfica* sp. nov. has

Figure 8. *Ledella elfica* sp. nov. Internal view (Holotype MNRJ 30482): **A,** right valve; **B,** left valve. External view (IBUFRJ 19364): **D,** right valve; **E,** left valve. Dorsal view (IBUFRJ 19363): **F,** right valve; **G,** left valve. Details: **C,** resilifer of the right valve. Prodissoconch (IBUFRJ 19324): **H,** overview; **I,** detail of the tip of the prodissoconch showing some granulation. Hinge teeth (IBUFRJ 19385): **J,** overview; **K,** detail of the tooth showing the tooth pustules. Scales A, B, D–G: 1 mm. C, H: 0.1 mm. I: 0.01 mm. J: 0.2 mm. K: 0.05 mm.

Diagnosis

Thick shell usually without anterior and rostral shoulders, but, when present, not prominent. Rostrum acutely rounded. Shallow rostral sinuosity.

Description

Shell oblong–elliptical, inflated, thick, inequivalve, inequilateral, H/L about 0.72; umbones prominent, opisthogyrous and inwardly directed. Antero-dorsal margin convex; anterior margin with a shoulder, rounded ventral margin. Ventral margin forming a shallow sinuosity leading to a rostrate posterior end; postero-dorsal margins lightly convex, sometimes with a weak shoulder near the end of the hinge plate. Smooth surface. Hinge plate interrupted by a deep, rounded resilifer. Posterior hinge plate longer and thinner than anterior one in adult specimens, and almost equal in juveniles. Width of the anterior and posterior rows of teeth occupying about 59% and 58%, respectively, of the total width of the hinge plate of the right valves and 61% and 57% to the anterior and posterior hinge plates, respectively, of the left valve. Shell microsculpture: the prodissoconch presents a weak granulation and the hinge teeth present pustules on the side that faces the umbones. Maximum shell length 2.02 mm. Prodissoconch height: 90 μm. Prodissoconch length: 118 μm. (Table 4)

Remarks

Ledella manati sp. nov. differs from *L. semen* in having less opisthogyrous umbones and a less acute rostrum than *L. semen*.

Ledella manati sp. nov. differs from *L. solidula* in having no concentric lines and a rostrum that is shorter and less acute than that of *L. solidula*.

Ledella manati sp. nov. differs from *L. ultima* in having no concentric lines and in presenting thinner teeth on the hinge plate and a more acute rostrum than *L. ultima*.

Ledella manati sp. nov. differs from *L. acinula* in having no concentric lines and in presenting a more acute rostrum and a less inflated posterior region of the shell than *L. acinula*.

Ledella manati sp. nov. differs from *L. orixa* in presenting less opisthogyrous umbones, a larger posterior region than the anterior region and a smaller anterior part of the hinge plate when compared to the posterior part.

Ledella manati sp. nov. is similar to *L. sublevis* in having a shallow rostral sinuosity, a smooth surface and a thick shell, but *L. manati* sp. nov. can be distinguished by its less acute rostrum and thinner hinge teeth.

Material examined (See supplemental document at: http://www.bioone.org/doi/suppl/10.4003/006.032.0201/suppl_file/Viegas_2014_Suppl_docs.pdf)

Etymology

Manati is a Caribbean word for the sea cow. Because sea cows are large, but possess a smooth contour that is similar to the shells of this species, we decided to name it *Ledella manati* sp. nov.

Distribution

Known from the Campos Basin, Rio de Janeiro, Brazil and for Trindade Island, Espírito Santo, Brazil.

Figure 9. *Ledella manati* sp. nov. Internal view (Holotype MNRJ 30483): **A,** right valve; **B,** left valve. External view (IBUFRJ 19435): **D,** right valve; **E,** left valve. Dorsal view (IBUFRJ 14673): **F,** right valve; **G,** left valve. Details: **C,** resilifer of the right valve. Prodissoconch (IBUFRJ 14637): **H,** overview; **I,** detail of the tip of the prodissoconch showing some granulation. Hinge teeth: **J,** overview; **K,** detail of the tooth showing the tooth pustules. Scales A, B, D–G: 1 mm. C, H: 0.1 mm. I: 0.02 mm. J: 0.2 mm. K: 0.01 mm.

Ledella manati sp. nov. is similar to *Ledella verdiensis* Allen and Hanna, 1989 in having a similar shell shape with a shallow rostral sinuosity, but it can be distinguished on the basis of having no strong concentric lines, a less high shell, a thicker hinge plate and a smoother postero-dorsal margin than *L. verdiensis*.

Ledella manati sp. nov. differs from *L. spocki* sp. nov. in having a shorter and less acute rostrum, with a rostral sinuosity that is about half the profundity as that of *L. spocki* sp. nov., and a longer and thinner anterior part of the hinge plate in relation to the shell size, but a shorter posterior part of the

hinge plate with no thickness difference to that of *L. spocki* sp. nov. The prodissoconch of *L. manati* sp. nov. is larger than that of *L. spocki* sp. nov. in both height and length, but there is no difference regarding its microsculpture.

Ledella manati sp. nov. differs from *L. legionaria* sp. nov. in having a longer and higher rostrum, with a rostral sinuosity that is about half the profundity as that of *L. legionaria* sp. nov. on the left valve, but about 66% less profound on the right valve. *Ledella manati* sp. nov. also has a longer and thinner anterior part of the hinge plate, but a shorter and thicker posterior part when compared to *L. legionaria* sp. nov. The hinge teeth occupy almost the same proportion of the hinge plate thickness in both species. Regarding the prodissoconch, they do not differ much in height and microsculpture, but the prodissoconch of *L. manati* sp. nov. is larger in length than the prodissoconch of *L. legionaria* sp. nov.

Ledella manati sp. nov. differs from *L. elfica* sp. nov. in having a shorter and less acute rostrum, with a rostrum sinuosity with about half the profundity as that of *L. elfica* sp. nov. Additionally, *L. manati* sp. nov. has a shorter and thicker posterior part of the hinge plate, but an anterior part with no difference in length or thickness compared to *L. elfica* sp. nov. Regarding the prodissoconch, they do not differ much in height and microsculpture, but the prodissoconch of *L. manati* sp. nov. is larger in length than the prodissoconch of *L. elfica* sp. nov.

DISCUSSION

One of the major problems associated with the traditional taxonomical approach is its subjectivity, which generates a relationship of dependency between the expertise of the taxonomist and the reliability of the taxonomical decisions. In general, the taxonomist notes, somewhat intuitively, many differences between the taxa he is working on. The main difficulty lies in making these differences clear to the readers. The morphometric approach itself does not solve this problem, because the taxonomist cannot handle the distinct measurements taken from all over the shell to determine which ones are relevant for discriminating among the taxa. A technique to reduce this myriad of potentially important variables to a manageable number of morphologically understandable factors is fundamental for obtaining objectivity in taxonomic

Table 4. Average values of the larval shell measurements and length of the adult shell. H(p) – Height of the prodissoconch I; *SD*, standard deviation; L(p), Length of the prodissoconch I; *n*, number of measured valves; L (s), Length of the adult shell; Min, Minimum value; Max, Maximum value.

	H(p)	*SD*	L(p)	*SD*	*n*	L (s)	Min	Max	*n*
Ledella spocki sp. nov.	0,071	0,005	0,073	0,004	20	1,674	1,336	1,937	60
Ledella legionaria sp. nov.	0,078	0,006	0,086	0,004	20	1,891	1,367	2,318	60
Ledella elfica sp. nov.	0,083	0,006	0,104	0,004	20	1,632	1,291	1,869	60
Ledella manati sp. nov.	0,091	0,004	0,118	0,003	20	1,708	1,401	2,027	60

work. The discriminant analysis is not the only statistical technique that is able to do this, but the method has a clear and simple mathematical basis (Klecka 1982). In our case, it reduced the original 44 variables to a few comprehensible factors.

Considering the right valves, the variables Prs, Lr and Hrd/Hrv, which are all related to the rostrum, were found to be critical characters in discriminating among these species. Moreover, a complementary set of variables (wap, wap/ahp, wpp, hpt, and hpt/W), which are all related to the hinge plate, was shown to be critical for discrimination. An additional set of morphological variables providing minor contributions completed the analysis. The rate of agreement between traditional taxonomy and this morphometric-based approach was 95%.

Analysis of the left valves produced similar results. Due to differences between right and left valves within the same specimen (inequivalve condition), a somewhat different set of variables was selected to maximize the distinction among the four taxa. The most relevant variables were: W, lhp/L, W/L, alm and R2. This set of variables carries information about the general shape of the valve, in contrast to the right valves analysis, which indicated different characters as being important. In the left valves analysis, the rate of agreement between traditional taxonomy and this morphometric-based approach was 88.33%.

The second set of analyses, which was based on hinge plate characters alone, revealed the importance of these features for distinguishing the four species: the rates of agreement between traditional taxonomy and this morphometric-based approach were 66.66% for the right valves and 64.16% for the left valves. This finding corroborates the importance of including the hinge plate structure in morphometric analysis, as stated by Benaim *et al.* (2011) in their study of *Yoldiella* species. This outcome is not surprising because hinge characters were important in the complete morphometric analyses performed earlier in this study. However, despite being highly important, the hinge plate characters alone have a lower rate of agreement between the traditional taxonomical approach and the morphometric approach than the analyses using the full set of morphometric variables. In other words, the variables that are associated with the shell (valve) shape are essential for producing a satisfactorily high rate of agreement between traditional taxonomy and this morphometric-based approach.

The larval shell of these species present some microsculpture. The prodissoconch I presents a weak granulation (Figs. 6H, 6I, 7H, 7I, 8H, 8I,9H and 9I) that cannot be perceived without the use of an electron microscope. The nepioconch cannot be clearly defined for these species, because either it is undifferentiated from the adult shell or it does not present a clear boundary with it.

Regarding the prodissoconch, it is also important to notice that the prodissoconch I of these species are smaller than expected for protobranchs (Gustafson and Reid 1986) and, since the prodissoconch II is not visible, it probably means that the eggs are brooded or lecithotrophic (Zardus and Martel 2002). Since no brooding was observed in the samples here analyzed, this suggests these species simply have rather small eggs.

The SEM images also revealed the presence of microsculpture on the hinge teeth of all four species described herein (Figs.6J, 6K, 7J, 7K, 8J, 8K, 9J and 9K). Malchus and Sartori (2012) describe similar teeth pustules in Nuculanidae, but the origin and function of these pustules are unknown (Malchus, pers. comm.). They may generate more attrition between the teeth, so that they are harder to slide, turning the shell harder to open by predators, but a study about its function must be done prior to defining its actual function. This information is not mentioned for the other *Ledella* species to which we compare our species, but we do not know if it is present or not, since the detection of this character is very specific and was not done for the other species yet.

CONCLUSIONS

The description of these four species improves the understanding of the biodiversity of protobranchs of the Campos Basis, contributes with some morphological aspects of *Ledella* species and corroborates the importance of including specific hinge plate details in the characterization of protobranch species.

ACKNOWLEDGMENTS

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The taxonomy and morphometry of squids in the family Loliginidae (Cephalopoda: Myopsida) from the Pacific coast of Mexico

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Abstract: The present research was a morphological and morphometric analysis of species from the family Loliginidae distributed in the Mexican Pacific and Gulf of California. These species are captured incidentally in shrimp fisheries and commercialized locally. Nevertheless, the number of species from the zone is uncertain, and there are numerous taxonomic problems in the group that limit the accurate identification of species and complicate the proper elaboration of the fisheries record. Morphological and morphometric analyses were performed using a canonical variable analysis (CVA) of 530 organisms. Six groups were separated a priori based on the shape of the funnel organ.

Based on the observed differences, four species were recognized: *Lolliguncula (Lolliguncula) panamensis* Berry, 1911, *Lolliguncula (Lolliguncula) argus* Brakoniecki and Roper, 1985, *Lolliguncula (Loliolopsis) diomedeae* (Hoyle, 1904), and *Doryteuthis (Amerigo) opalescens* (Berry, 1911). Two additional forms were recognized and named the following: *Lolliguncula* sp. 1, and *Lolliguncula* sp. 2. Three canonical variables (CV) explained 95.4% of variability among the groups in the analysis results. Variance percentages related to CV1, CV2, and CV3 were 61.9, 19.5, and 13.9%, respectively. A MANOVA analysis supported the statistically significant differences among the squid groups (Wilk's lambda = 0.023; $F_{(75.2447)}$ = 38.6; $P < 0.0000$). In accordance with the analyses developed in this research, it is evident that there are morphological and morphometric characteristics that indicate a greater diversity of taxa are present compared to the list of species previously reported off the Pacific coasts of Mexico.

Key words: Mexican Pacific, discriminant analysis, cephalopod, funnel organ,

Squids from the family Loliginidae are represented by approximately 47 species grouped in ten genera and nine subgenera (Brakoniecki 1986, Nesis 1987, Vecchione *et al.* 2005, Jereb *et al.* 2010). Species in this family distribute from very shallow waters in bays and estuaries, over grass flats and coral reefs, to depths over 700 m, primarily in warm to temperate waters world-wide (Roper *et al.* 1984, Jereb *et al.* 2010). Loliginids are important in trophic webs of marine ecosystems as predators of small fishes and invertebrates and prey for demersal and pelagic fishes of commercial importance and marine mammals (Fields 1965, Recksiek and Frey 1978, Markaida and Hochberg 2005, Galván-Magaña *et al.* 2007).

A total of approximately 20 loliginid species are captured in coastal or near-shore artisanal fisheries, sometimes referred to as small-scale fisheries, and industrial fisheries worldwide. However, only four (*Doryteuthis (Amerigo) gahi* (d'Orbigny, 1835), *D. (Amerigo) pealeii* (Lesueur, 1821), *D.*

(*Amerigo*) *opalescens* (Berry, 1911) and *Loligo reynaudii* d'Orbigny, 1841) are identified to species level in fisheries statistics (FAO, 1998). All the other species tend to be pooled in the genus *Loligo* Lamarck, 1798 in arrival notifications and global statistics (FAO 1998, Vecchione *et al.* 1998). The absence of detailed descriptions that guarantee accurate species identification is the principal cause of this problem, making management of these resources difficult. In Mexico, loliginids commonly caught by artisanal fisheries and as by-catch in shrimp trawl fisheries off the Mexican Pacific coast and in the Gulf of Mexico (Roper *et al.* 1984, Cardozo and Valdivieso 1988, Roper *et al.* 1995, Barrientos and García-Cubas 1997, Alejo-Plata *et al.* 2001, Alejo-Plata 2002, Sánchez 2003). Commercial fishing occurs during the entire year and demand continues to increase; therefore, defining the taxonomic status of the group through an exploration of alternative tools than those currently used is urgent. Thus, discrimination

between species will allow researchers to perform the biological studies necessary for the implementation of accurate management plans based on an individual approach of fisheries in Mexico.

The global taxonomic basis of the family Loliginidae remains complex and incomplete, particularly concerning specific and generic divisions (Vecchione *et al.* 2005, Cardoso and Hochberg, 2013). Therefore, a number of authorities reached consistency on the urgent necessity to conduct a comprehensive taxonomic review of this family (Voss 1977, Roper *et al.* 1983, Guerra 1992, Voss *et al.* 1998, Anderson 2000a, 2000b, Vecchione *et al.* 2005, Jereb *et al.* 2010).

Off the Pacific coast of Mexico and in the Gulf of California, five species have been reported: *Lolliguncula* (*Lolliguncula*) *panamensis* Berry, 1911; *Lolliguncula* (*Lolliguncula*) *argus* Brakoniecki and Roper, 1985; *Lolliguncula* (*Loliolopsis*) *diomedeae* (Hoyle, 1904); *Doryteuthis* (*Amerigo*) *opalescens* (Berry, 1911); and *Pickfordiateuthis vossi* Brakoniecki, 1996.

A few published studies of the group have been based on the spatio-temporal distribution of paralarvae (Okutani and McGowan 1969), records of stomach contents in fishes and marine mammals (Markaida and Hochberg 2005, Galván-Magaña *et al.* 2007), or general descriptions and distribution maps (Young 1972, Okutani 1980, Roper *et al.* 1984, Alejo-Plata 2002, Sánchez 2003). Because of the absence of taxonomic studies, there is considerable confusion on species identification and the total number of valid species in the region. In a study of invertebrates from the Gulf of California, *Loliolopsis chiroctes* Berry, 1929 and *L. diomedeae* are listed as separate species (Hendrickx and Brusca 2005), although *L. chiroctes* is a junior synonym of *L. diomedeae* (Voss 1971). Moreover, in 1980, Brakoniecki described *Lolliguncula tydeus* based on organisms from Central America and south Mexico. In a subsequent study, he considered *L. tydeus* as a junior synonym of *L. panamensis* (Brakoniecki 1986). Despite this correction, several authors have considered *L. tydeus* as a valid species (Cardozo and Valdivieso 1988, Alejo-Plata *et al.* 2001).

A taxonomic revision of the species in the family Loliginidae with distribution off the Mexican Pacific coasts and in the Gulf of California was performed in this survey based on the funnel organ, which presented noticeable visual differences among specimens. In addition, the shape of the mantle and fins, fin length proportions, tentacle lengths, arm lengths, tentacular club lengths, and sucker number in buccal membrane ribs, among other characters, were analyzed. In previous studies on squids, little taxonomic importance has been provided to the funnel organ for species discrimination, and it has only been referred to occasionally in the species description of a few families: Enoploteuthidae (McGowan and Okutani 1968, Roper 1964) and Loliginidae (Burgess 1967, Roper and Vecchione 2001). For some species from the tropical Eastern Pacific, the funnel organ shows an inverted "V" shape (Brakoniecki 1980, Barrientos-MacGregor 1985, Brakoniecki and Roper 1985, Cardozo and Valdivieso 1988, Barrientos and García-Cubas 1997, Alejo-Plata *et al.* 2001, Alejo-Plata 2002, Sánchez 2003). To confirm the funnel organ's effectiveness as a discriminating characteristic, a morphological and morphometric analysis was performed using multivariate analyses. These techniques have been employed with effective results on squid species discrimination using beaks (Martínez *et al.* 2002), statoliths (Neige 2006), and morphometric variables (Barón and Ré 2002).

MATERIALS AND METHODS

Field procedures

In total, 530 specimens were analyzed. Of this total, 404 were collected as by-catch in shrimp trawls off the Pacific coast of Mexico (Fig. 1) from the northern Gulf of California to the state of Oaxaca, including the western coast of the Baja California Peninsula (off Bahia Magdalena, Baja California Sur). The remaining 126 individuals analyzed were specimens housed in the cephalopod collection at the Santa Barbara Museum of Natural History (SBMNH), California, U.S.A. All specimens were preserved in 70% alcohol but had different treatments during fixation (Table 1).

Laboratory procedures

The specimens were identified and grouped according to the funnel organ. The funnel organ is an glandular structure fused to the internal surface of the funnel, generally a single W-shaped form in octopods and a dorsal inverted V-shaped component with opposed ventral oblong components in decapods (Roper *et al.* 1984) (Fig. 2C).

Morphological and morphometric analyses were performed to test the effectiveness of funnel organ discrimination. Sixteen measurements were obtained for each individual using a digital caliper to the nearest 0.01 mm (Roper and Voss 1983). The measurements included: mantle length (ML); mantle width (MW); middle mantle length (MML); right fin length (RFL); left fin length (LFL); fin width (FW); head length (HL); head width (HW); eye diameter (ED); arm I length (IAL); arm II length (IIAL); arm III length (IIIAL); tentacle length (TL); tentacle club length (TCL); funnel width (FuW); and funnel length (FL) (Figs. 2A, 2B). The lengths of both fins were included because a high percentage of *Lolliguncula* (*Lolliguncula*) *panamensis* specimens presented differences between left and right fin length.

The measurements were provided as proportions of mantle length (ML) using the mantle proportion length index proposed by Roper and Voss (1983). ML was considered because it is one of the few measurements that remain
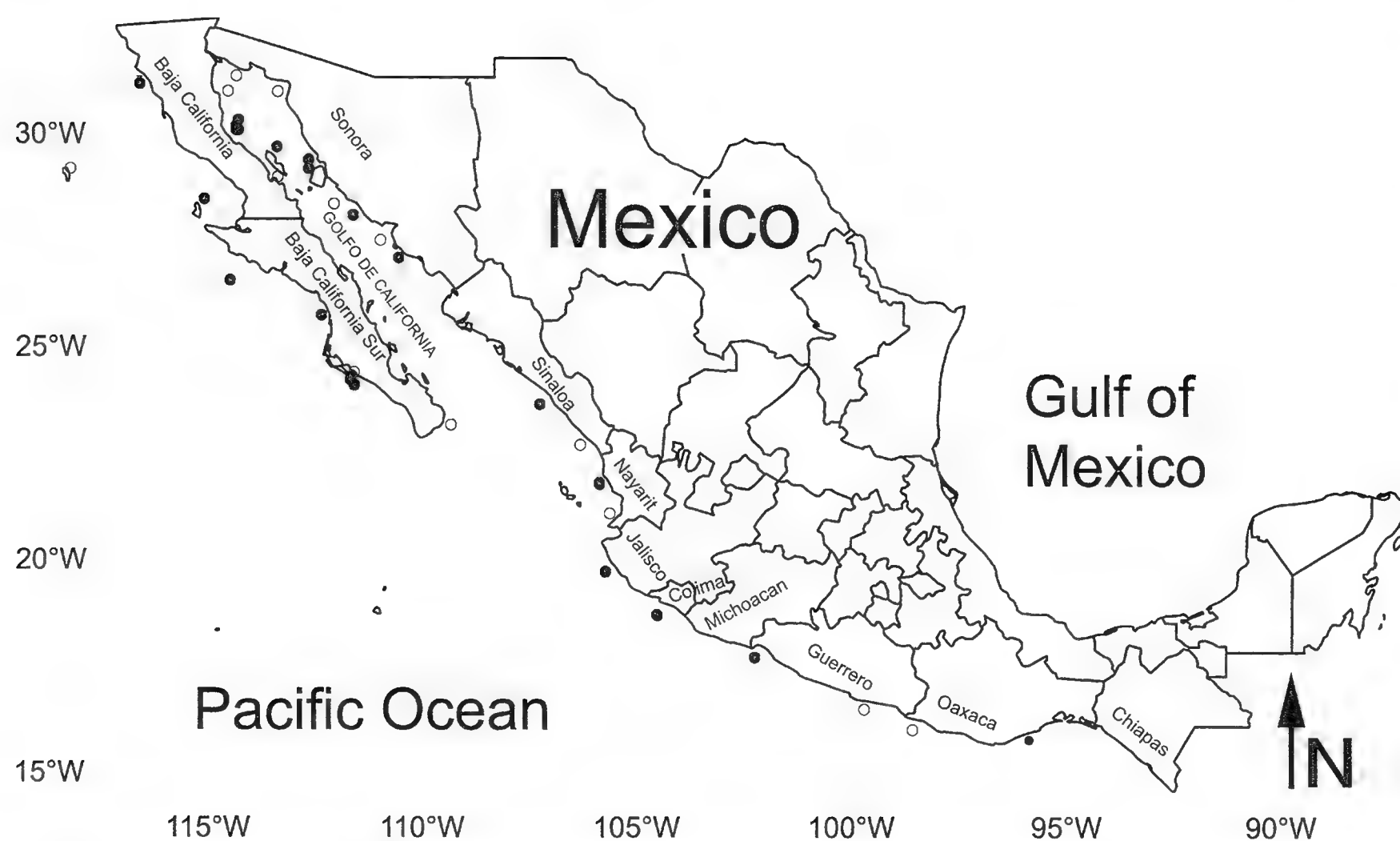
Figure 1. The location of sampling points off the Pacific coasts of Mexico and in the Gulf of California. Symbols: (●) Solid circles indicate present study; and (○) empty circles indicate specimens from the collection of the Santa Barbara Museum of Natural History (SBMNH).

consistent, independent from material condition, cause of death, or posterior treatment (Fields 1965). Additionally, an angular (x+0.01) transformation, was performed to all variables to stabilize the variance of the average during the proportion study, whereas it approached the variables in a normal distribution (Sokal and Rohlf 1985). This matrix was used for the multivariate analyses. A separate sex analysis was not shown because the sample size in five of the six analyzed taxa was relatively small. However, to avoid possible bias, hectocotylus measurements were not considered in this study. The hectocotylus (H) is a character with high taxonomic value in these species; nevertheless, given it is larger in size than the rest of the arms, its inclusion in the analysis would generate sex separation affecting the rest of the variables.

Statistical data analysis

A canonical variable analysis (CVA) was performed using 530 individuals and 15 variables (excluding mantle length). Statistical significance of the differences between groups was determined using Wilks' (λ) Lambda statistic, whose value when equal or close to 0 indicates a good discrimination model in contrast to 1, which represents a nonpredictable model (Tabachnick and Fidell 1996). Moreover, the Mahalanobis (D^2) quadratic distances matrix was used for morphometric dissimilarity pattern analyses among the examined groups through an unweighted pair group method with the arithmetic mean analysis (UPGMA). The analyses were performed using the Statistica 8.0 program.

Voucher specimens

From the identified organisms, reference specimens were deposited in the collection of cephalopods housed at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) collection located in La Paz, Baja California Sur, Mexico.

RESULTS

Species accounts

Lolliguncula (Lolliguncula) panamensis **Berry, 1911**

Synonyms: *Lolliguncula tydeus* Brakoniecki, 1980.

Funnel organ description: The dorsolateral patches were narrow and elongated with a crest on the superior section. The ventral patches were elongated and wide and thin to the inferior ends with a pronounced protuberance on the middle section. The superior section terminated in a triangular shape and was inferior with rounded tips. This section showed a fold on the internal section that covered 80% of its size. The funnel valve was wide with an identically sized superior fold (Fig. 3A).

Lolliguncula (Lolliguncula) argus **Brakoniecki and Roper, 1985**

Synonyms: none

Funnel organ description: The dorsolateral patches were elongated and wider on the superior section. The ventral patches were elongated and narrow, the superior section was markedly pointed, and the inferior ends were rounded. The funnel organ showed a fold on the internal section that covered 40% of its size. The funnel valve was narrow with a pair of folds on its lateral sections that ended at the patches level (Fig. 3C).

Lolliguncula (Loliolopsis) diomedeae **(Hoyle, 1904)**

Synonyms: *Loliolopsis chiroctes* Berry, 1929

Funnel organ description: The dorsolateral patches were elongated, wide on the superior section and thin on the inferior. The ventral patches were thin and elongated and slightly wider in the middle section. The superior section terminated in a triangular shape and the inferior ends were rounded. The funnel organ presented a fold on the internal section that covered approximately 70% of the patch size. The funnel valve was wide with an identically sized fold on the inferior section and another on the superior section that extended throughout the funnel width (Fig. 3B).

Table 1. Species, location, latitude and longitude, realized cruises list off the Pacific coast of Mexico and in the Gulf of California, treatment, and number of specimens. Abbreviations, cruises: UAENIP = Unidad Académica Escuela Nacional de Ingeniería Pesquera; CIBNOR = Centro de Investigaciones Biológicas del Noroeste; CUCSUR = Centro Universitario de la Costa Sur; SBMNH = Santa Barbara Museum of Natural History. Abbreviations, species: Do = *Doryteuthis (Amerigo) opalescens*; La = *Lolliguncula (Lolliguncula) argus*; Ld = *Lolliguncula (Loliolopsis) diomedeae*; Lp = *Lolliguncula (Lolliguncula) panamensis*; Lsp1 = *Lolliguncula* sp.1, and Lsp2 = *Lolliguncula* sp.2.

Location	Latitude /Longitude	Cruise	Treatment	No. of specimens	Species					
					Do	La	Ld	Lp	Lsp1	Lsp2
Sinaloa	23°65'N, 107°14'W	UAENIP-2009	96% Ethanol	44			44			
Nayarit	21°79'N, 105°75'W	UAENIP-2009	96% Ethanol	50			1	49		
Baja California (Gulf of California)	29°69'N, 113°33'W/ 30°09'N, 114°25'W	CIBNOR-2008	10% Formalin, then 70% Ethanol	107		2	22	83		
Sonora	27°09'N, 110°46'W/ 28°09'N, 111°53'W	CIBNOR-2008	10% Formalin, then 70% Ethanol	50			2	48		
Guerrero	17°68'N, 102°08'W	CIBNOR-2009	96% Ethanol	44			44			
Oaxaca	15°74'N, 95°63'W	CIBNOR-2011	96% Ethanol	26			26			
Baja California (Pacific coast)	31°19'N, 116°58'W/ 28°48'N, 115°06'W	CIBNOR-2011	96% Ethanol	3	2		1			
Baja California Sur (Pacific coast)	26°56'N, 114°43'W/ 25°75'N, 112°26'W	CIBNOR-2010	96% Ethanol	34	2	1	30	1		
Jalisco	19°70'N, 105°61'W	CUCSUR-2009	96% Ethanol	24			24			
Colima	18°69'N, 104°39'W	CUCSUR-2009	96% Ethanol	22			22			
SBMNH	various	various	10% Formalin, then 70% Ethanol	126	12	5	16	33	6	54
TOTALS				524	16	8	232	214	6	54

***Doryteuthis (Amerigo) opalescens* (Berry, 1911)**

Synonyms: *Loligo stearnsi* Hemphill, 1892

Funnel organ description: The dorsolateral patches were elongated, had a subtriangular shape, and rounded tips. The ventral patches were elongated and divided and wider in the middle section. The superior and inferior ends were rounded. It had a wide fold on the internal section that covered approximately 70% of the patch size without extending to the ends. The funnel valve was wide with a fold on the superior section that ended at the dorsal patch level (Fig. 3D).

***Lolliguncula* sp. 1**

Funnel organ description: The dorsolateral patches were wide and rounded on the superior section and pointed and curved at the inferior ends. The ventral patches were wide on the superior section and united in a "U" shape with thin inferior ends and were notoriously separated. It did not show folds in the middle section. The funnel valve was wide and thin without folds (Fig. 3E).

***Lolliguncula* sp. 2**

Funnel organ description: The dorsolateral patches were semi-circular and slightly wider in the superior section. The ventral patches were elongated and wide with an irregular shape, which was wider on the superior section with a small narrowing on the middle section that became wider again and finished into a tip. The superior section was narrow and pointed and showed a thin fold on the internal section that covered approximately 80% of its size. The funnel valve was narrow and not much folded (Fig. 3F).

Morphometry

We identified and grouped the 530 organisms into six groups (four species and two forms) based on funnel organ shape (Fig. 3). There were strong differences among the examined groups based on the results of the morphometric analyses. According to the canonical variable analysis, the initial three canonicals variables explained 95.4% of the variability among the groups. The variance percentages explained by CV1, CV2, and CV3 were 61.9, 19.5, and 13.9%, respectively (Fig. 4). The MANOVA analysis showed statistically significant differences among the squid groups (Wilk's lambda = 0.0239; $F_{(75.2447)}$ = 38.6; $P < 0.0000$). The correct classification functions obtained for the six groups showed a matrix with a 77.0% correct assignment average, which, in general terms, indicates the high taxonomical integrity of the groups. Nevertheless, the *Lolliguncula* sp. 1 group presented a low (33.3%) correct assignment percentage, although this had only been confused with *Lolliguncula (Loliolopsis) diomedeae*. A greater sample size should better clarify these results. The highest assignation percentage was observed for *Lolliguncula (Lolliguncula) panamensis* with 97.1% (Table 2).
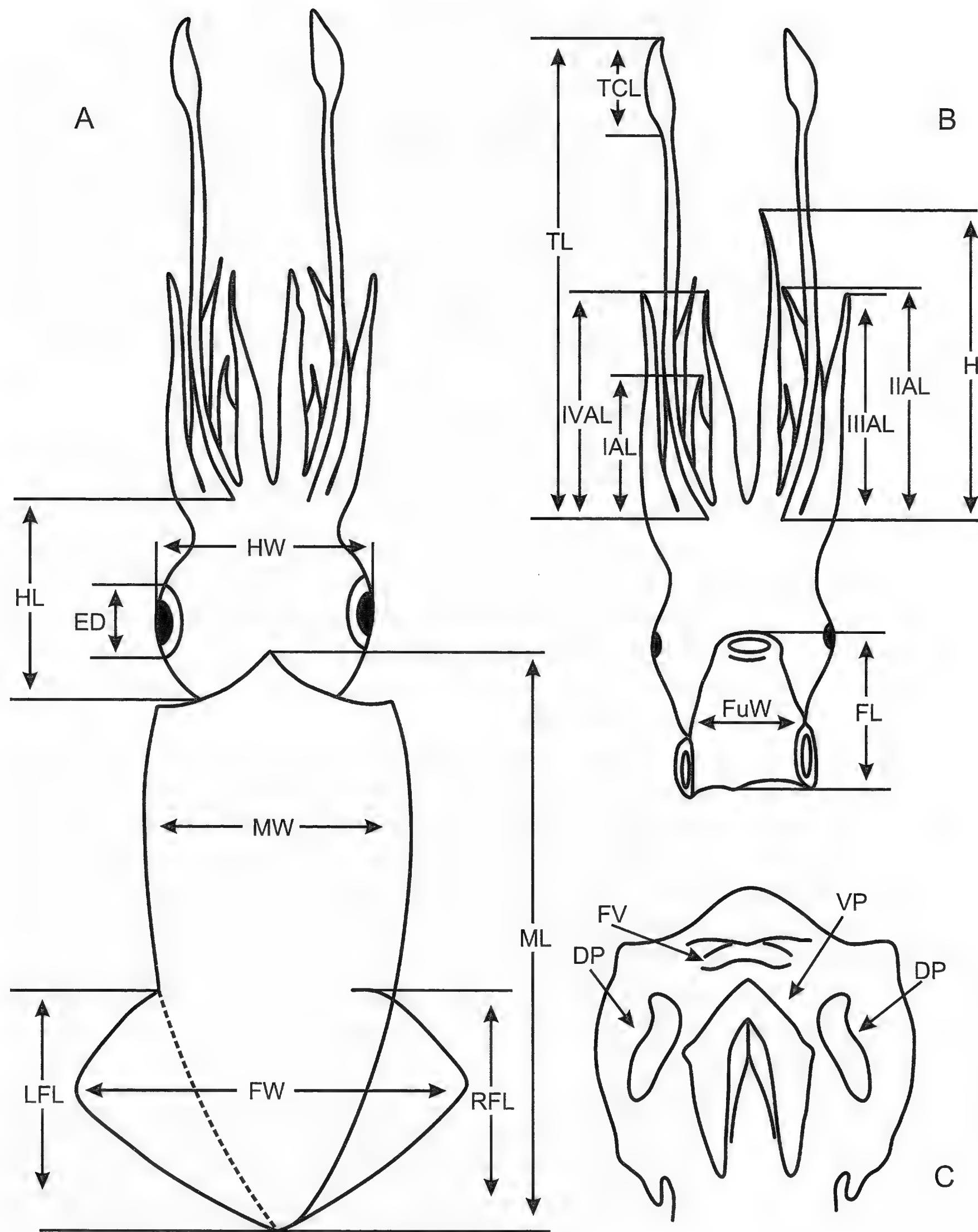
Figure 2. A diagram of the measurements. **A,** Dorsal view, **B,** ventral view, and **C,** structure of the funnel organ. Abbreviations: **DP,** dorsal pad, **ED,** eye diameter, **FL,** funnel length, **FuW,** funnel width, **FV,** valve funnel, **FW,** fin width, **H,** hectocotylus, **HL,** head length, **HW,** head width, **IAL,** arm I length, **IIAL,** arm II length, **IIIAL,** arm III length, **IVAL,** arm IV length, **LFL,** left fin length, **ML,** mantle length, **MML,** middle mantle length, **MW,** mantle width, **RFL,** right fin length, **TCL,** tentacular club length, **TL,** tentacle length (Roper and Voss 1983), and **VP,** ventral pad.

The obtained CVA value distribution showed a discrimination of two groups (explained by CV1): one group comprised *Lolliguncula panamensis* and the other by the rest of the species and the forms. The variables that showed high loading and CV1 included middle mantle length, right fin length, left fin length, tentacular club length, head width, and funnel width. Particularly, *L. panamensis* differed from the other species and the two forms by longer middle mantle and

fin lengths. Given that unlike the rest of the species and the two forms, this species presents larger, wide and rounded fins. Although there was an overlap of shapes from CV2 and CV3, it was possible to observe agglomerations associated with each species. Thus, without considering *L. panamensis*, it is possible to distinguish *L. diomedeae* and the form *Lolliguncula* sp. 2 from the rest of the species and the form *Lolliguncula* sp. 1 in CV2. With respect to the observed loadings, the most important characteristics from the aforementioned differences were tentacle length, tentacular club length, head width, mantle width, arm lengths III, fin width, middle mantle length, right fin length, and left fin length. The results obtained from CV3 indicated that *L. argus*, *Doryteuthis opalescens*, and the form *Lolliguncula* sp. 1 were distinguished from other groups mainly because of the following characteristics: head width, fin width, head length, mantle width, tentacle length, and tentacular club length (Table 3).

The morphological similarity pattern obtained from Mahalanobis distances among the centroids of each group indicated that *Lolliguncula argus* was the most morphometrically divergent species whereas *Lolliguncula* sp. 1 and *Lolliguncula diomedeae* were among the most similar groups (Fig. 5). A great similarity between these two groups was also observed for the two classification functions, which misclassified one-half of the individuals of form *Lolliguncula* sp. 1 with *Lolliguncula diomedeae* (Table 2). However the distance topology does not represent an evolutionary history of this group, it is merely an assessment of the studied characters.

DISCUSSION

The loliginids are a diverse family of cephalopods (47 spp. and ten genera; Jereb *et al.* 2010), they represent a group with severe problems in systematics, which include the absence of taxonomic stabilization at higher levels (subgenera,
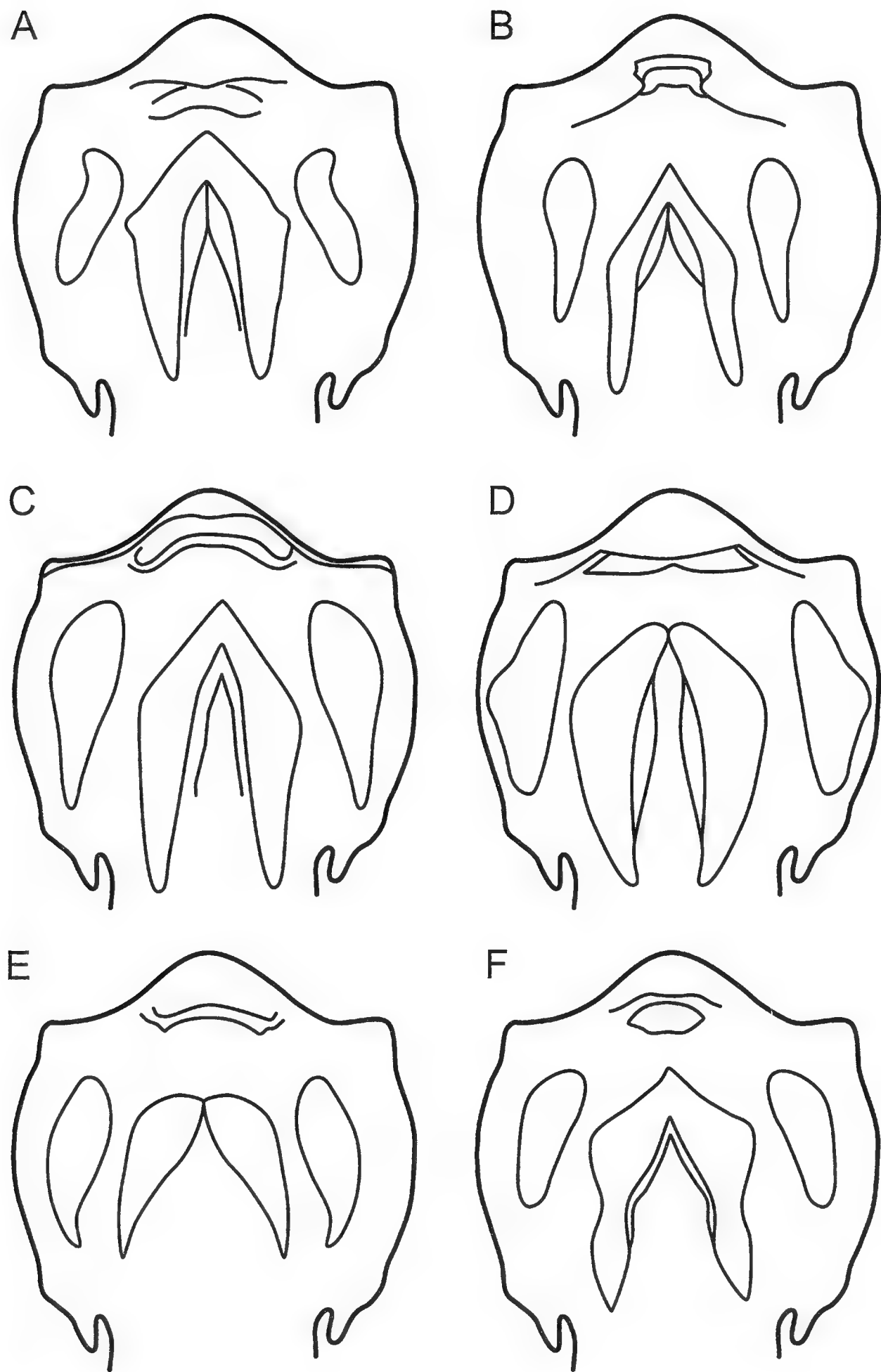
Figure 3. Diagram of the funnel organ differences: **A)** *Lolliguncula* (*Lolliguncula*) *panamensis*; **B)** *Lolliguncula* (*Loliolopsis*) *diomedeae* (SBMNH-60093 female ML 70.5 mm); **C)** *Lolliguncula* (*Lolliguncula*) *argus* (SBMNH-60043 female ML 61 mm); **D)** *Doryteuthis* (*Amerigo*) *opalescens*; **E)** *Lolliguncula* sp. 1 (SBMNH-64383 female ML 80.7 mm); and **F)** *Lolliguncula* sp. 2 (SBMNH-60083 female ML 44.3 mm).

Figure 4. The distribution of the scored frequencies obtained from the CVA in the six groups. **A,** CVA 2 x CVA 1; **B,** CVA 3 x CVA 1. Symbols: ○ *Doryteuthis* (*Amerigo*) *opalescens*; ▲ *Lolliguncula* (*Lolliguncula*) *argus*; ■ *Lolliguncula* (*Lolliguncula*) *panamensis*; ● *Lolliguncula* (*Loliolopsis*) *diomedeae*; △ *Lolliguncula* sp. 1, and + *Lolliguncula* sp. 2.

genera, and subfamily) and problems at the species level, including cryptic species and the possibility of natural hybridization among neighbor species (Brakoniecki 1986, Vecchione *et al.* 2005, Cardoso and Hochberg 2013). In the current research, loliginid specimens from the western coast of Mexico were analyzed using morphologic and morphometric analyses. Organisms from the species *Pickfordiateuthis vossi* were not captured as this species inhabits very shallow areas near the coast (Jereb *et al.* 2010). However, this species can be easily identified by modified tentacles, which are similar in appearance to pairs of arms IV, although they do not show a tentacular club. The posterior fin lobes extend beyond the posterior end of the mantle and do not join

posteriorly, unlike the remaining species from the region. Moreover, the buccal membrane does not have suckers, and the funnel organ has an inverted "U" shape (Brakoniecki 1996).

The funnel organ was a determining taxonomic characteristic for discrimination of the species and the two forms. This character has the advantage of not presenting differences regarding sex. Important differences regarding size, shape, and width of the ventral and dorsal patches were observed. In addition, these differences were easily distinguishable in organisms preserved in formaldehyde, but they were not visible in fresh organisms and organisms preserved in alcohol. In these cases, the use of methylene blue as an aid to emphasize the structures is recommended.

Our multivariate analysis supported the existence of four species, *Lolliguncula panamensis*, *Lolliguncula argus*, *Lolliguncula diomedeae*, and *Doryteuthis opalescens*, and two forms, *Lolliguncula* sp. 1 and *Lolliguncula* sp. 2. These last

Table 2. The classification matrix of species in the family Loliginidae. Abbreviations: Lp = *Lolliguncula* (*Lolliguncula*) *panamensis*; La = *Lolliguncula* (*Lolliguncula*) *argus*; Ld = *Lolliguncula* (*Loliolopsis*) *diomedeae*; Lsp1 = *Lolliguncula* sp. 1; Lsp2 = *Lolliguncula* sp. 2; and Do = *Doryteuthis* (*Amerigo*) *opalescens*.

Species	% Correct	No. of specimens classified						
		Do	La	Ld	Lp	Lsp1	Lsp2	Total
Do	75.00	12	2	1	1	0	0	16
La	75.00	0	6	2	0	0	0	8
Ld	94.39	0	0	219	1	1	11	232
Lp	97.19	0	1	1	208	1	3	214
Lsp1	33.33	0	0	4	0	2	0	6
Lsp2	90.74	0	0	0	5	0	49	54

shapes were assigned to the genus *Lolliguncula* according to the proposed criteria of Hoyle (1904), Berry (1911), Brakoniecki (1980 and 1986), and Brakoniecki and Roper (1985) and based on the following characteristics: body shape, fin size and shape, and a buccal membrane.

A discriminant analysis showed that using 15 variables, species could be classified accurately 77.6% on average: *Lolliguncula panamensis* (97.2%), *L. argus* (75%), *L. diomedeae* (94.4%), *Doryteuthis opalescens* (75%), and *Lolliguncula* sp. 2 (90.7%). In this regard, the high percentage of correct classification in the discriminant analysis suggests that the form *Lolliguncula* sp. 2 is morphometrically different from *L. panamensis* and *L. diomedeae*. These results and the observed differences in the funnel shape indicate that the form *Lolliguncula* sp. 2 could belong to a new undescribed species. In addition, the individuals grouped in the form *Lolliguncula* sp. 1 were weakly supported (33.3%) and based on the observed confusion in the classification matrix, the grouping was more similar to *L. diomedeae*. Nevertheless, the low

percentage of classification of this species might have been affected by the small sample size. An increase in both sample size and number of sampling localities may bring better results. The proportions in the hectocotylus size of the form *Lolliguncula* sp. 1 were greater (115–125%) than that reported for *L. diomedeae* (76–107% ML), and for the form *Lolliguncula* sp. 2 (37–106%). Unfortunately, the hectocotylus was not evaluated in detail for the species and the two forms. Collateral analysis by reviewing the hectocotylus in a deeper detail, statolith geometric morphometrics, genetic analyses based on mitochondrial DNA, should be done on the specimens deposited in the Santa Barbara Museum of Natural History.

The observed confusion may result from the high intraspecific morphological variability, which increases with individuals of both sexes and different sizes. In this study, from the 15 analyzed variables, 11 were positive variables for species and two forms discrimination. This highly overlapping of characteristics among loliginid species has been widely

Table 3. The Factor Structure Matrix Correlation Variables of CVA of Loliginidae off the Pacific coast of Mexico. Variables with a larger contribution are emphasized in bold-faced font.

Measurement	CV1	CV2	CV3
Fin length (right)	0.171480	**0.385246**	**0.241742**
Fin length (left)	**0.465680**	-0.235947	-0.040737
Mantle length (dorsal)	**0.386146**	-0.201109	-0.067915
Middle mantle length	**-0.769860**	0.263458	-0.044251
Fin width	**0.246934**	0.092860	0.000141
Funnel length	0.133937	0.171190	0.080246
Funnell width	0.129915	**0.311112**	**-0.392916**
Head length	0.171110	0.137354	**0.277466**
Head width	**0.252350**	**0.490012**	**0.454467**
Eye diameter	0.159098	0.022863	-0.145151
Arm I length	0.734900	0.111758	0.183470
Arm II length	0.045018	0.088165	0.025650
Arm III length	0.123661	**0.381721**	0.037506
Tentacle length	0.111437	**0.794937**	**-0.224416**
Tentacular club length	**0.273415**	**0.547080**	**-0.215554**
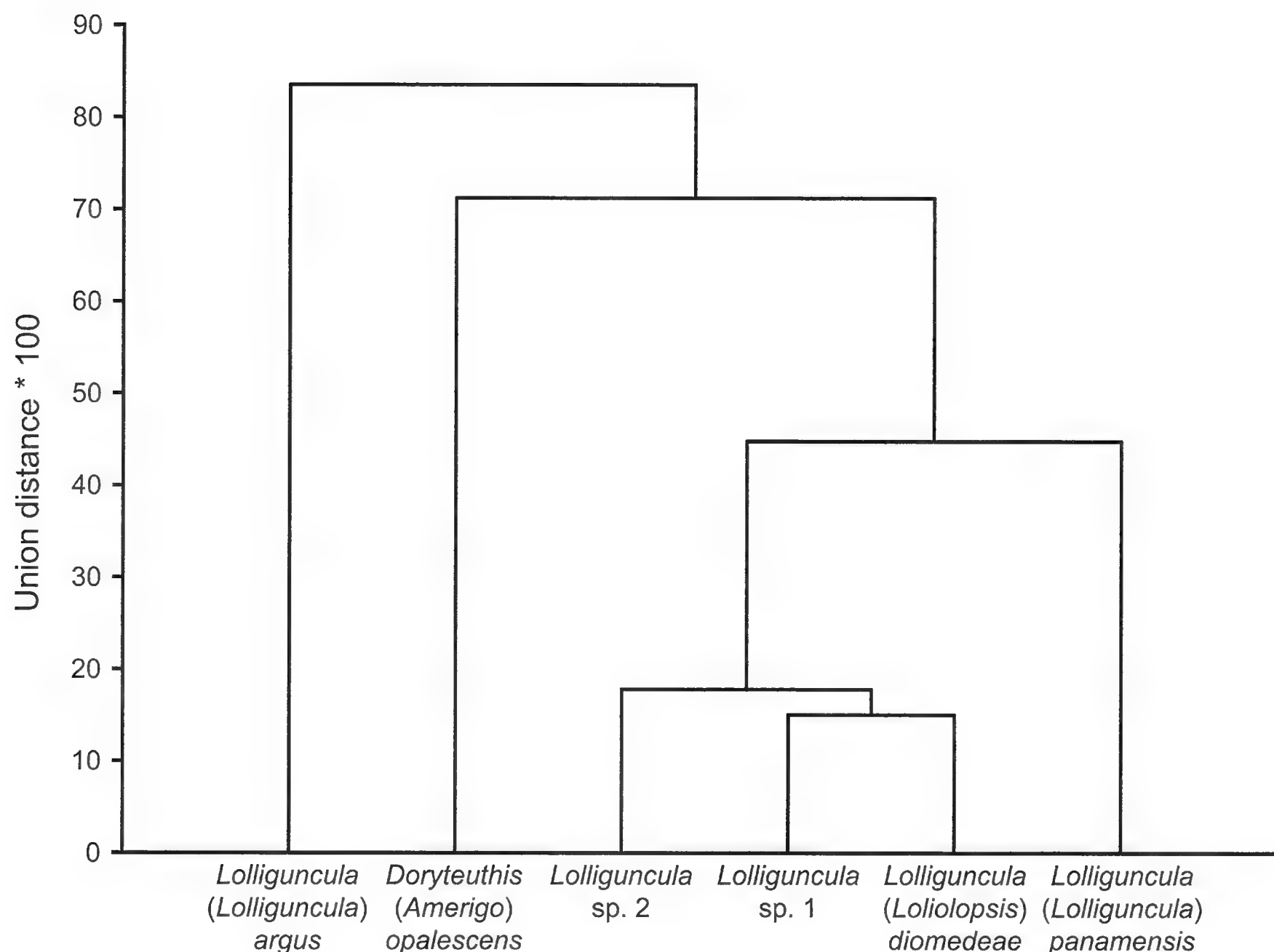
Figure 5. Dendogram based on the distances D2 of Mahalanobis and union UPGMA of *Doryteuthis (Amerigo) opalescens*, *Lolliguncula (Lolliguncula) argus*, *Lolliguncula (Lolliguncula) panamensis*, *Lolliguncula (Loliolopsis) diomedeae*, *Lolliguncula* sp. 1, and *Lolliguncula* sp. 2, off the Pacific coasts of Mexico and in the Gulf of California.

commented on by several authors (Anderson 2000a, 2000b, Vecchione *et al.* 2005).

Multivariate techniques have been shown to enhance species discrimination (Martinez *et al.* 2002). According to our analysis, high loading variables, particularly, *Lolliguncula panamensis*, differ from the others by longer middle mantle and fin lengths. Whereas *L diomedeae* and the form *Lolliguncula* sp. 2 differ in tentacle length, tentacular club length and head width from the remaining species. Lastly, *L. argus*, *D. opalescens*, and the form *Lolliguncula* sp. 1 differ from the other groups because of the following characteristics: head width, fin width, and head length. Based on the results of this research, although recent loliginid species identification guides (Roper *et al.* 1995, Sánchez 2003, Jereb *et al.* 2010) use the percentage of fin length to discriminate species, this criterion was not an important variable. Moreover, more than 50% of specimens showed one fin larger than the other; hence measurements of both fins were obtained from organisms in the present study.

Additionally, the presence of sexual dimorphism in the group makes identification more complex, thus requiring separate descriptions for males and females. In males the hectocotylus has a great taxonomic value to separate species (Brakoniecki 1986); however, in females there is no analogous organ with such taxonomic value. *Lolliguncula argus*

males share a cylindrical mantle shape with small and narrow fins with *L. diomedeae*, but differ from *L. diomedeae* and the remaining species by presenting the hectocotylus in the left ventral arm. *Lolliguncula diomedeae* and *L. panamensis* males differ from one another in the arms. *Lolliguncula diomedeae* has two modified ventral arms: the left arm is proportionally larger (76-107% ML) than that of *L. panamensis* (42-69% ML). Moreover, the length relationships of mantle-mantle width from these species show obvious differences: *L. diomedeae* is "thinner" than *L. panamensis*; in other words, its body has a more fusiform shape (Sánchez 2003). Lastly, in *Doryteuthis opalescens*, the mantle is cylindrical with large and triangular fins.

Hoyle's 1904 description of *Loliolopsis diomedeae* was based on a female captured in Acapulco, Guerrero, Mexico, 1891. This description showed that more information was necessary for the accurate and complete identification of the species, according to the species description criteria of Roper and Voss (1983). In Hoyle's 1904 original description, the specimen's mantle length was not considered. This measurement is perhaps the most important in a description because comparisons can be performed with other organisms and proportions of the different body structures can be obtained from the specimens. This absence of basic data was also evident in the description of *Lolliguncula panamensis* (Berry 1911). Berry only analyzed three females (85, 101 and 105 mm ML) when describing the species, and the data presented only referenced the location where the organisms were captured (Panama) and the location of the type specimens (Stanford University Museum).

Further Brakoniecki (1980) described *Lolliguncula tydeus* as a new species based on specimens in the collections of the Rosenstiel School of Marine and Atmospheric Science of the University of Miami, which were catalogued as *Loliolopsis diomedeae* (Hoyle, 1904). This author analyzed males and females of different sizes (26-53 ML), determined a detailed morphological description of the internal and external structures, and separated the species based mainly on differences in the hectocotylus shape. However, after examining larger collections in 1986, Brakoniecki concluded that *L. tydeus* was the male of *L. panamensis* and was therefore a junior synonym of *L. panamensis*.

For the tropical eastern Pacific, Roper *et al.* (1994) presented an illustrated identification guide for four species: *Doryteuthis* (*Amerigo*) *opalescens*, *Lolliguncula* (*Loliolopsis*) *diomedeae*, *Lolliguncula* (*Lolliguncula*) *argus*, and *Lolliguncula* (*Lolliguncula*) *panamensis*. Nonetheless, the authors did not provide a basis for a precise discrimination of genera and subgenera. Moreover, the diagnostic characteristics were based on adult specimens; therefore, young specimen identification remained confusing.

Based on the results of the present and previous research on the group, we recognize that there are at least five loliginid species in the Mexican Pacific, *Lolliguncula* (*Lolliguncula*) *panamensis*, *Lolliguncula* (*Lolliguncula*) *argus*, *Lolliguncula* (*Loliolopsis*) *diomedeae*, *Doryteuthis* (*Amerigo*) *opalescens*, and *Pickfordiateuthis vossi* (Roper *et al.* 1995; Jereb *et al.* 2010). From the four identified species in this research, *Lolliguncula panamensis* and *L. diomedeae* are widely distributed in the Eastern Tropical Pacific. The distribution of *Doryteuthis opalescens*, with temperate affinity, is reported from the south western coast of Baja California Peninsula, Mexico to southeastern Alaska, U.S.A. *Lolliguncula argus* is restricted to the northwest Mexican Pacific. Recently there was a report on the presence of a population on the west coast of Baja California Sur and in the Gulf of California, approximately 950 km off its known distribution range (Granados-Amores *et al.* 2013). To validate the species treated herein as *Lolliguncula* sp. 1 and *Lolliguncula* sp. 2 a detailed morphological description, including the hectocotylus description is needed. In addition, field evaluations are required to precisely determine their distribution ranges.

According to the analysis developed in the present research, there were morphologic and morphometric characteristics that indicated a greater diversity of species compared to what was previously reported for the Mexican Pacific. However, it is necessary to increase the sample size for some species and explore innovative and powerful tools such as geometric morphometry in statoliths, which has provided good results for separating teuthid, octopod and cuttlefish species, including some loliginid species of the Mediterranean Sea (Dommergues *et al.* 2000, Lombarte *et al.* 2006, Neige 2006). Genetic and molecular analyses can also be useful for cryptic species identification (Vecchione *et al.* 2005).

ACKNOWLEDGMENTS

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RESEARCH NOTE

First report of the invasive slug *Boettgerilla pallens* Simroth, 1912 (Boettgerillidae) in the United States

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Abstract: *Boettgerilla pallens* Simroth, 1912 is reported for the first time in the United States. Identification was based on combined morphological and molecular data (mitochondrial *cytochrome c oxidase* I and 16S rRNA sequences) of a specimen collected in a retail garden center in northern California. We provide some background information on the biology and ecology of the slug and discuss the pest status of the species.

Key words: worm slug, European pest gastropod, subterranean slug, potted plants

Herein we report the first record of the European invasive slug *Boettgerilla pallens* Simroth, 1912 in the United States. The specimen was collected under a potted plant in a retail garden center in San Mateo in northern California (37°34′18″N; 122°18′57″W) on 27th January 2013. Other gastropod species collected with *B. pallens* at the garden center were *Arion hortensis* agg., *Deroceras laeve* (Müller, 1774), *Deroceras reticulatum* (Müller, 1774), *Lehmannia valentiana* (Férussac, 1821), and *Oxychilus cellarius* (Müller, 1774).

We confirmed the identification of *Boettgerilla pallens* using both morphological and molecular means. The dissected specimen has been deposited at Department of Malacology, Academy of Natural Sciences in Philadelphia under catalogue number ANSP A23428. Fragments of the mitochondrial *cytochrome c oxidase* subunit I (COI) and 16S rRNA genes were amplified and sequenced following the methods of Barr *et al.* (2009) using the Folmer *et al.* (1994) COI primer set LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′), and the 16S primer set 16S-1 (5′-CGCCTGTTTAACAA-AAACAT-3′) and 16S-2 (5′-ACGCCGGTTTGAACTCAG-ATC-3′). COI and 16S sequences of the San Mateo specimen were deposited in GenBank (Benson *et al.* 2013) under accession numbers KF849344 (631 bp) and KF849350 (355 bp), respectively. When compared to other sequences from specimens collected in Germany and the United Kingdom (KF849345–KF849349, KF849351–KF849355), our slug was 100% identical

to *B. pallens* for both gene fragments. When compared to data on GenBank, the next best matches (87% for each gene) were to Helicarionidae samples. These results are, therefore, congruent with the morphological identification.

Boettgerilla pallens is a slender, medium sized slug up to 50 mm in length (Reise *et al.* 2000). It has a translucent appearance and tends to be yellowish gray, pale gray, or bluish gray (Fig. 1) with a contrasting darker colored head. The mantle of the slug is approximately one third the length of the body and the dark keel runs from the end of the mantle to the tip of the tail (Grimm *et al.* 2009). When fully extended it resembles an earthworm, hence its common name, the worm slug. *Boettgerilla pallens* is a burrowing species, often utilizing earthworm tunnels. It has been found at depths of 60 cm but it is thought that a small portion of the population remains at the ground surface under leaves, stones, and wood. It feeds on earthworm feces, eggs of other gastropods, carrion, fungal hyphae, roots, decaying vegetation, and detritus (Forsyth 2004). The worm slug is known from a wide range of habitats including gardens, grassland, woodland (deciduous and coniferous), greenhouses, and nurseries (Grimm *et al.* 2009, Reise *et al.* 2000) and is tolerant of a range of soil types, soil water content, and soil pH (De Wilde *et al.* 1983).

The native range of the species is thought to be the Caucasus in south-eastern Europe. Over the past sixty years the slug has spread rapidly throughout Europe, the former USSR and into the Americas, with first records from North America (British Columbia, Canada) in 1998 (Reise *et al.*

Figure 1. Specimen of *Boettgerilla pallens* Simroth, 1912 collected in San Mateo, California. Length of specimen is ~15mm. (Figure shown in color only in the electronic version).

2000) and from South America (Colombia) in 2000 (Hausdorf 2002). Although there is no evidence that *Boettgerilla pallens* is economically important, this may be an artifact of the primarily subterranean nature of the species, which likely makes it difficult to detect. Reise *et al.* (2000) also suggested that this uncertainty regarding the pest nature of the worm slug is compounded by the fact that other known pest species often co-occur with it (as was the case with our record; see above). Regardless of the pest status of *B. pallens,* its burrowing nature and presence in garden centers and nurseries has likely facilitated the spread of the species in potted plants. It is, therefore, very probable that the species exists in other parts of the United States as well and we hope that this note will stimulate research on and surveys for this understudied slug.

ACKNOWLEDGMENTS

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RESEARCH NOTE

Notes on the natural history and ecology of *Inflectarius magazinensis* (Pilsbry and Ferriss, 1907) (Gastropoda: Polygyridae), the Magazine Mountain Shagreen

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Abstract: *Inflectarius magazinensis* (Pilsbry and Ferriss, 1907), the Magazine Mountain Shagreen, is an endemic land snail found only on Magazine Mountain, Arkansas, U.S.A. This species was given the status of threatened in 1989, but was removed from the threatened list by the United States Fish and Wildlife Service in June 2013 due to apparent stability of the population within its known habitat. *Inflectarius magazinensis* is a rupicolous species inhabiting talus areas of Savanna sandstone. Populations occur from approximately 670 m to 790 m on the north and west sides of Magazine Mountain. Our study was designed to gather information regarding basic natural history and ecology knowledge for this species. We examined both known localities and sites where there were no historical records for the presence of *I. magazinensis* but suitable habitat was present. We found that this species is restricted to only the north- and west-facing slopes of Magazine Mountain. Due to its restricted habitat preferences, the total range of this species amounts to approximately nine hectares on the upper slopes of the mountain. Because *I. magazinensis* is endemic to Magazine Mountain and restricted to upper elevations, it should be monitored on a regular basis. Due to levels of uncertainty in terms of climate change, pollution, and disturbance (both natural and anthropogenic), habitat health should be taken into consideration as a metric used for monitoring the stability and long-term viability of this species.

Key words: food habits, Arkansas, Magazine Mountain, rare land snail

The land snails of Arkansas have been overlooked and are poorly represented in the scientific literature. The first papers concerning Arkansan Gastropoda examined species distributions across the entire state, as well as within certain regions in the state (Sampson 1893, Pilsbry 1903, Pilsbry and Ferriss 1906). Most studies have been compilations for certain localities within the state (Hubricht 1967, Hubricht 1972, Gordon 1980, Coles and Walsh 1999, Walsh and Coles 2002) with the exception of Solem (1976) who commented on the genus *Polygyrid* Because so little research has been done on the molluscan fauna of Arkansas, basic population and life history information, especially for rare species, is nonexistent.

Inflectarius magazinensis (Pilsbry and Ferris, 1907) is a rupicolous (*i.e.*, rock inhabiting) species of land snail endemic to Magazine Mountain, Arkansas. It was originally listed as threatened in the late 1980's (United States Fish and Wildlife Service 1989) based on a preliminary status report (Caldwell 1986), but has since been proposed for removal from the threatened status (Davidson 2012) and subsequently removed (Arkansas Ecological Services Field Office 2013) largely because of apparent population stability, investigations into

habitat requirements, and proposed continuation of monitoring (Caldwell *et al.* 2010, United States Fish and Wildlife Service 2013). However, certain aspects of natural history and basic ecology remain unknown for this species.

This study was initiated to define *Inflectarius magazinensis'* range and obtain natural history information for use in the Revised Recovery Plan. Since it so closely resembles two other species, *I. edentatus* (Sampson, 1889) and *I. inflectus* (Say, 1821), they were included in the study to document the distinctness of *I. magazinensis*. The ranges of the three species and some habitat information are given in Hubricht (1985). *Inflectarius inflectus* is common throughout the eastern United States in a variety of habitats. *Inflectarius edentatus* is primarily found on wooded slopes north of Magazine Mountain in eastern Oklahoma, northwestern Arkansas, and southern Missouri (Pilsbry 1903, Pilsbry and Ferriss 1907, Hubricht 1972).

The primary objective of this study was to delineate and map the range of *Inflectarius magazinensis*. Additionally, we wished to determine the ecology and habitat associations (*i.e.*, vegetation and land snail cohorts) of this species by examining sites where the species was known to be present and sites where

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Table 1. Mean greater diameter measurements and numbers for *Inflectarius* species found during this study.

Species	Number	Mean Greater Diameter
Inflectarius edentatus (Sampson, 1889)	34	11.65
Inflectarius inflectus (Say, 1821)	69	10.31
Inflectarius magazinensis (Pilsbry and Ferris, 1907)	45	12.76
Undescribed *Inflectarius* sp.	41	11.12

conditions were suitable but no historical records of its presence were available. A secondary objective was to document the reproductive habits of this species as well as to record food sources, feeding behaviors, and principal activity periods.

Survey periods were 8–19 May 2007, 10–12 July 2007, 30 September–5 October 2007, and 5–14 May 2008. On Magazine Mountain, 40 study plots (20 north side and 20 south side) were established to record habitat parameters such as vegetation, biotic, abiotic, and physical attributes of known and potential sites. The surrounding areas of Poteau, Short, and Sugar Loaf Mountains were surveyed for potential habitat. Mount Nebo (535 m elevation) and Petit Jean Mountain (360 m elevation) were chosen for more intense surveys due to the presence of core habitats, similar to that of Magazine Mountain (839 m elevation).

All study sites were located in the central portion of the Arkansas River valley ecoregion (Level III) and the Scattered High Ridges and Mountains ecoregion (Level IV) (Woods *et al.* 2004). Vegetated Savanna Sandstone rock talus is a dominant feature of this region (Vere 1986) and is the preferred habitat of *Inflectarius magazinensis* (Caldwell 1986).

Study plots were circular with a 10 m radius (314 m² in area). Within each plot, data concerning the following variables was collected: percent canopy cover, duff accumulation (*i.e.*, cm of organic soil horizon), percent bare ground, percent leaf litter, percent rock, percent coarse woody debris (CWD), aspect, slope, tree species present, shrub species present, forest community type, basal area (BA), and snail associates. Percent canopy cover, percent bare ground, percent leaf litter, percent

rock, rock characteristics, and percent coarse woody debris (by decay class) was determined by ocular estimation.

Tree and shrub vegetation was sampled on both north and south slope talus of Magazine Mountain. Vegetation was sampled using the point-quarter method (Cottam and Curtis 1956). This method was modified by having a 4 x 4 m sampling plot for shrubs (Caplenor 1968). Trees were defined as having a diameter at breast height (DBH) equal to or greater than 10 cm. Any woody stem plant having a diameter less than 10 cm was considered a shrub. Data collected were used to determine importance values (IV) (*i.e.*, the summation of relative frequency, relative density, and relative coverage [scale 0–300]) of each species.

Taylor digital max/min thermometers were set out 5 May and removed 11 May 2007. This yielded recordings for six, 24 hour periods. Daily maximum and minimum temperatures were determined for ground surface, within rock crevices, and for the atmosphere (50 cm above the ground). Fifteen thermometers were placed on the south slope talus where two were used to obtain ground and atmospheric temperature and 13 were used for rock crevice temperature. Fourteen thermometers were placed on the north slope talus where two were for recording ground and atmospheric temperature and 12 were for recording rock crevice temperature.

Terrestrial gastropod species present at each plot were determined by hand collection of larger shells, timed searches (20 person minutes) within microhabitats present (*i.e.*, leaf litter, rock talus, CWD, exfoliating bark present on standing snags and downed woody material, hollow trees and forks of trees where detritus had accumulated, damaged trees excreting sap, and cliff line features), and through laboratory searches of one liter bags filled with appropriate microhabitat substrate material. A minimum of one bag was collected from each quarter section, equaling four bags per plot. All litter was inspected for larger snails before being placed in bags to assure no collection of *Inflectarius magazinensis*. Night surveys were conducted to assess activity periods and to observe feeding behavior. The following measurements were taken in millimeters on each of the three target species when
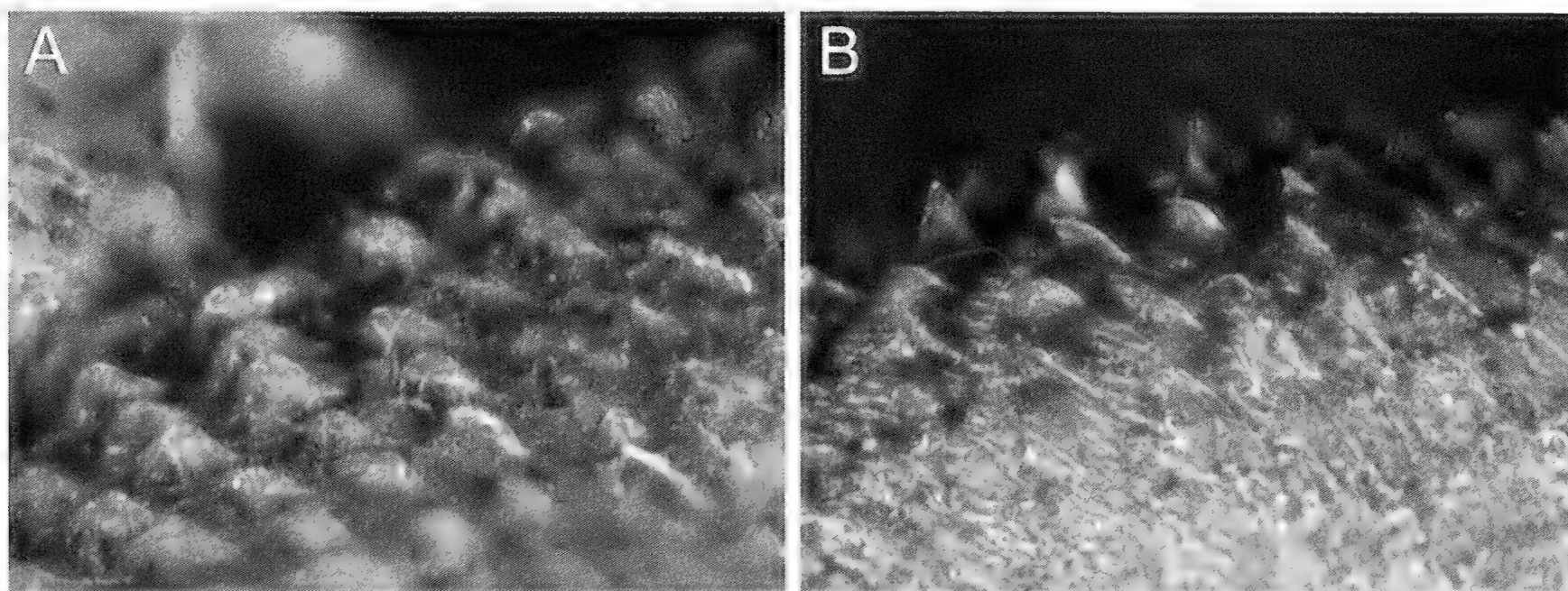
Figure 1. Periostracal processes of **A**, *Inflectarius magazinensis* compared to **B**, *Inflectarius inflectus*. Total magnification 250X. (Color shown in electronic version only).

possible: greater diameter (GD) (measured from the aperture all the way across the shell), lesser diameter (LD) (diameter perpendicular to the GD), umbilicus diameter (UD), and whorl count (W) (every full whorl to the right of the apex).

Individuals of *Inflectarius inflectus* in which aperture teeth are reduced can be very similar to *I. magazinensis*. *Inflectarius magazinensis* is the largest of the three species, followed by *I. edentatus*, and *I. inflectus* (Table 1). Periostracal processes of *I. inflectus* and *I. magazinensis* are substantially different from one another (Fig. 1). *Inflectarius magazinensis* has semi-lunar processes while *I. inflectus* processes are more delta- or tooth-shaped. Periostracal processes of *I. edentatus* and *I. magazinensis* are identical. However, these two species are not sympatric. The sizes of *I. edentatus* and *I. magazinensis* are comparable and the shells are indistinguishable.

No *Inflectarius magazinensis* were found outside of Magazine Mountain. Populations occur on the upper slopes of the north and west sides of Magazine Mountain within a 120 m range. Populations were documented to occur on the north-facing slopes just below the cliff line at approximately 670 m and on the west-facing slopes at approximately 790 m. Incorporating all known localities, the total range of this species is approximately nine hectares in area.

Within the tree layers of the study site, *Tilia americana*, *Acer saccharum*, and *Fraxinus americana* were found only in north slope talus (Table 2). *Quercus rubra* had the highest IV of all tree species found in the north slope talus (IV = 1.09) but was fourth highest in the south slope talus (IV = 0.21) (Table 2). Within the shrub layers of the north and south slope, *Ribes cynosbati* was found only in the north slope talus and had the second highest IV of any north slope talus shrub (Table 2).

The number of max/min temperature readings taken for each temperature comparison between slopes are as follow: rock crevice north slope 114, rock crevice south slope 131; atmospheric north slope 15, atmospheric south slope 13; and ground north slope 12, ground south slope 12. An analysis of variance comparison of rock crevice, atmospheric, and ground temperatures between north and south slopes found no significant differences for maximum temperatures. However, significant differences were found for minimum temperatures. Ground minimum temperatures on the north slope were 3.1 °C lower than on the south slope ($P = 0.01$). Atmospheric minimum temperatures on the north slope were 2.9 °C lower than on the south slope ($P = 0.005$). Rock crevice temperatures were found 2 °C cooler on the north slope ($P < 0.0001$).

Euconulus fulvus (Müller, 1774), *Vertigo milium* (Gould, 1840), *V. tridentata* Wolf, 1870, *Glyphyalinia lewisiana* (G. H. Clapp, 1908), and *Millerelix dorfeuilliana* (I. Lea, 1838) were found only on the south slope talus where *Inflectarius magazinensis* does not occur (Table 3). *Neohelix divesta* (Gould, 1848), *Zonitoides arboreus* (Say, 1816), and immature *Succineidae* sp.

Table 2. Importance values for trees and shrubs found in study plots on both the north and south facing slopes of Magazine Mountain.

Species	North Slope	South Slope
Trees		
Acer saccharum	0.08	
Asimina triloba		0.06
Carya spp.	0.32	0.95
Celtis sp.		0.07
Fraxinus americana	0.05	
Juglans nigra	0.09	0.20
Nyssa sylvatica	0.18	0.32
Ostrya virginiana	0.28	0.01
Quercus alba		0.61
Quercus alba	0.24	
Quercus muehlenbergii	0.11	
Quercus rubra	1.09	0.21
Quercus velutina		0.20
Robinia pseudoacacia	0.14	0.19
Sassafras albidum	0.18	0.14
Tilia americana	0.22	
Shrubs		
Acer rubrum		0.04
Aesculus glabra	0.02	
Amelanchier arborea	0.08	0.02
Aralia spinosa	0.07	
Asimina triloba		0.42
Carya spp.	0.13	0.24
Castanea pumila	0.05	0.04
Celtis sp.		0.05
Cercis canadensis		0.23
Chionanthus virginicus	0.02	
Cornus florida		0.10
Corylus americana		0.18
Dioscorea villosa	0.02	
Fraxinus americana	0.02	
Hydrangea arborescens	0.04	
Nyssa sylvatica	0.15	0.30
Ostrya virginiana	0.62	
Quercus alba		0.13
Quercus rubra	0.16	0.03
Ribes cynosbati	0.47	
Robinia pseudoacacia	0.16	0.26
Rubus flagellaris		0.14
Rubus occidentalis	0.05	
Rubus sp.	0.02	
Sassafras albidum	0.29	0.30
Smilax spp.	0.02	0.12
Tilia americana	0.04	
Ulmus sp.	0.02	
Vaccinium sp.	0.36	
Viburnum prunifolium		0.05
Vitis sp.	0.17	0.36

Table 3. Abundance (ABD) and relative frequencies (RF) of land snails collected from leaf litter sample bags on north and south slopes of Magazine Mountain. Boldface indicates micro-snail (< 5 mm in diameter). Numbers in parentheses represent abundances.

Species	North Slope		South Slope	
	ABD	RF	ABD	RF
Anguispira strongylodes (Pfeifer, 1854)	26	0.049	2	0.006
Carychium exile H.C. Lea, 1842	32	0.061	43	0.130
Discus patulus (Deshayes, 1830)	17	0.032	21	0.063
Euconulus fulvus (Müller, 1774)			10	0.030
Gastrocopta contracta (Say, 1882)	8	0.015	4	0.012
Gastrocopta pentodon (Say, 1821)	41	0.078	12	0.036
Glyphyalinia indentata (Say, 1823)	5	0.009	8	0.024
Glyphyalinia lewisiana (Clapp, 1908)			26	0.078
Glyphyalinia wheatleyi (Bland, 1883)	31	0.058	27	0.081
Guppya sterkii (Dall, 1888)	37	0.070	38	0.114
Haplotrema concavum (Say, 1821)	25	0.047	9	0.027
Hawaiia minuscula (A. Binney, 1840)	1	0.001	3	0.009
Helicodiscus parallelus (Say, 1817)	10	0.019	14	0.042
Inflectarius inflectus (Say, 1821)	39	0.074	6	0.018
Mesomphix cupreus (Rafinesque, 1831)	37	0.070	4	0.012
Millerelix dorfeuilliana (I. Lea, 1838)			1	0.001
Mesodon zaletus (A. Binney, 1837)	50	0.095	10	0.030
Neohelix divesta (Gould, 1848)	3	0.006		
Patera binneyana (Pilsbry, 1899)	19	0.036	1	0.001
Punctum minutissimum (I. Lea 1841)	47	0.089	30	0.090
Paravitrea multidentata (A. Binney, 1840)	14	0.027	1	0.001
Succineidae (immature)	1			
Strobilops aeneus Pilsbry, 1926	3	0.006	6	0.018
Stenotrema labrosum (Bland, 1862)	17	0.032	3	0.009
Striatura meridionalis (Pilsbry and Ferriss, 1906)	41	0.078	12	0.066
Ventridens brittsi (Pilsbry, 1892)	11	0.021	1	0.001
Vertigo gouldi (A. Binney, 1843)	11	0.021	27	0.081
Vertigo milium (Gould, 1840)			3	0.009
Vertigo tridentata Wolf, 1870			1	0.003
Zonitoides arboreus (Say, 1816)	1	0.001	1	0.001
TOTALS	527	0.995	324	0.993

were the only species, outside of *I. magazinensis*, that occurred exclusively on the north slope talus (Table 3).

Inflectarius magazinensis were found during night surveys feeding on oak catkins, algal-covered rocks, and decaying *Quercus alba* (white oak) leaves. There were three documented feeding events, which represent the first report on the food habits of *I. magazinensis*.

During this study, breeding and egg-laying occurred in early May concomitant with the spring rains. No live individuals were found on the surface during night surveys or

under the leaf litter in July 2008. Night surveys conducted in October 2007 could not document surface activity but live individuals were found under the leaf litter. No egg masses were found in the October survey.

Prolonged drought or concomitant warming of temperatures, either through natural cycles or anthropogenically influenced (*i.e.*, climate change), could adversely affect species of land snails that occur at high elevations, such as *Inflectarius magazinensis* (Pearce and Paustain 2013). Most critically, nesting sites and egg masses may be compromised due to desiccation and lead to extinctions. Simulated laboratory experiments have shown this to be a consequence of climatic warming (Baur and Baur 1993). While adults may feed within the talus, surface feeding may be necessary to replenish calcium stores for egg deposition. If the habitat and food sources have been altered because of changes to climate, this raises questions about the long-term viability of this species. While there is still a great deal of uncertainty around the science of climate change, most models point toward dire consequences for biodiversity and skyrocketing extinction rates (Bellard *et al.* 2012). For a species with narrow ranges, like *I. magazinensis*, forecasts such as this are very grim. Consequently, it would be wise to err on the side of caution and continuously monitor and protect *I. magazinensis* habitat in order to maintain biodiversity and ecological integrity.

Our results are corroborated by the vegetative communities and land snail associates that are found on the north and south slopes of Magazine Mountain. Our data shows a much more mesic community present on the north slope (Table 2) where *Inflectarius magazinensis* occurs and suggests an inability to survive in drier conditions. Land snail associates present on the south slope reflect these drier conditions and belong to genera that tend to be adapted to more harsh circumstances (Nekola 2010). This indicates that changes in moisture regimes may lead to substantially altered communities, both in vegetation and land snail associates, as well as possible extinctions in rare species like *I. magazinensis*.

Previous studies hypothesized that other polygyrids were somewhat generalist in their food habits (Foster 1936, Blinn 1963). The most detailed research on food habits of any woodland polygyrid has been conducted on *Triodopsis platysayoides* (Brooks, 1933), another rupicolous federally threatened species (Dourson 2008). That species is a generalist and has been reported to feed on a variety of items, including dead camel crickets. *Inflectarius magazinensis* most likely mirrors these generalist food habits, therefore, food source is probably not limiting to this species. Woodland polygyrids likely sort food habits out by size cohorts (Foster 1936, Blinn 1963). Of course, micro-snails (< 5 mm in diameter) within the duff may feed on completely different items. We speculate this to be the case for juvenile *I. magazinensis*.

Because *Inflectarius magazinensis* is a species for which very little is known, systematically monitoring populations is a necessity in order to ensure population stability. While very little may be able to be done to protect this species from alterations induced by climate change, appropriate monitoring would allow for changes to be rapidly detected and necessary steps taken to preserve this species. A more proactive approach may be to monitor ecosystem health (*e.g.*, land snail community structure, vegetative communities, micro-climates) rather than focusing on one single species. Examining trends within the community would help predict potential perturbations and declines in habitat health that could adversely affect target species.

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RESEARCH NOTE

Arm abnormality in *Octopus hubbsorum* (Mollusca: Cephalopoda: Octopodidae)

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Abstract: *Octopus hubbsorum* Berry, 1953 occurs in a wide region of the oriental tropical Pacific. This is the principal target species in the octopus fisheries in Mexico. A female with abnormally bifurcated second arm was collected from an artisanal fishery in Puerto Angel, Oaxaca, Mexico.

Key words: Octopus, Oaxaca, bifurcation, *Hox* genes

Cephalopods are a diverse group of highly derived molluscs, including nautiluses, squids, cuttlefish, and octopuses. Regeneration in cephalopods has been observed and reported by several workers. Toll and Binger (1991) documented different cases of arm anomalies in octopoda, and González and Guerra (2006) mentioned cases of bilateral hectocotilization in squids and cuttlefish. The most common anomalous condition of the arms of octopuses is the bifurcation or polyfurcation of the arms, particularly at the tips (Toll and Binger 1991).

Parona (1900) described bifurcation in an arm of *Eledone moschata* Lamarck, 1798 and the first left arm of *Octopus vulgaris* Lamarck, 1798 in the Mediterranean Sea. Kumpf (1960) described a male specimen of *O. briareus* Robson, 1929 with the third left arm divided into two branches. It showed a well-developed web between these divisions as found at the bases of the normal arms in the species. In an extreme case reported by Okada (1965 in Toll and Binger 1991), seven of the eight arms of a specimen of *Octopus* sp. were involved in polycotomous branching resulting in a total of ninety terminal branches. Palacio (1973) reported cases of double unilateral hectocotylization in *O. vulgaris* and *O. selene* Voss, 1971. Gleadal (1989) studied the anatomy of octopuses with seven arms, with special reference to the arrangement of brachial nerves. The first case of true hexapody among the Octopoda, resulting from bilateral agenesis of one arm pair, was described by Toll and Binger (1991) for a male *Pteroctopus tetracirrhus* della Chiaje, 1830. The same authors also reported the first case of decapody in octopuses for a male *O. briareus* and concluded that both conditions apparently result from developmental anomalies of the embryonic arm anlagen.

Knowledge of octopus from the central Eastern Pacific (20°N–15°S) is limited to a very few species and biological characteristics. In fact, data on octopods from the central and south Mexican Pacific are very scarce and fragmentary; *Octopus hubbsorum* Berry, 1953 is the principal target species in the octopus fisheries (Alejo-Plata *et al.* 2009, López-Uriarte and Ríos-Jara 2009, Domínguez-Contreras *et al.* 2013). Records on disease conditions are scarce (Pascual *et al.* 2006) and oil anomalous conditions are absent. The objective of this study was to describe bifurcation in an arm of the *O. hubbsorum*.

A female *Octopus hubbsorum* with second arm bifurcated was found among a sample of 115 specimens collected by scuba diving (5–10 m depth) as part of an artisanal fishery in Mexico, El Faro Puerto Angel (15°39′N, 96°31′W) during January to October 2012. The specimens were examined and identified using the Roper *et al.* (1995)'s taxonomic key together with the Berry (1953)'s original description.

Mantle length (ML), total length (TL), and total body weight (BW) were taken. Maturity was determined by presence of mature oocytes in females. The octopus was formalin-fixed and deposited in the cephalopod collection at the Universidad del Mar at Oaxaca, Mexico.

The specimen conforms well to all of the morphological features typically associated with the species. The main measurements of this female were 275 g body weight and 75 mm ML. This female was immature. Its stomach was full. Except the arm bifurcations, this female did not show any other type of anomaly (Fig. 1A).

Length of the arm before the split was 70 mm; the arm was divided into two branches, the longest branch (215 mm) and shorter (140 mm) had a localized large knob (approximately 5 mm diameter) in the later margin, and regeneration of arm tip (Table 1). An unusual feature was the presence of a

Figure 1. A, Dorsal view of *Octopus hubbsorum*; **B, C,** Oral view of brachial crown, shows second bifurcated arm.

Table 1. Morphological measurements of the specimen of *Octopus hubbsorum* with arm abnormality.

Biometrics	Measurement (mm)
Total length	380
Mantle length	75
Mantle width	45
Head width	38
Arms length (to base of split)	70
Arms length of left branch	215
Arms length of right branch	140
Depth of web	31
Arm order	2, 4, 3, 1

suckers, distally from the center of the junction, is composed of one sucker across. The last row of suckers on the main stem has five suckers across the arm. On each of the divided sections the paired sucker arrangement found on the main stem is present (Figs. 1B–C). All remaining arms are normal. Figure 2 shows the body weight-mantle length relationship of the 115 specimens in the sample. The open square represents the female with abnormalities in the arm.

The arm branching observed in *Octopus hubbsorum* does not appear to be the result of any arm injury; similar results are described by Kumpf (1960) in *O. briareus*. Toll and Binger (1991) suggested a possible relationship of polyfurcation of arm tips in the Octopoda with regenerative processes in amphibian limbs. It is conceivable that additional cases of true arm agenesis in octopods have gone undiagnosed because of the frequency of routine injury-induced arm loss. Also, the regenerative process associated with arm repair is normally easily visualized until the arm has regained almost its entire length.

Homeobox genes are a large family of similar genes that direct the formation of many body structures during early embryonic development. Genes in the homeobox family are involved in a wide range of critical activities during development, include directing the formation of limbs and organs along the anterior-posterior axis and regulating the process by which cells differentiate; mutations in these genes are responsible for a variety of developmental disorders. For example, mutations in the *Hox* group of homeobox genes typically cause limb malformations. The expression of *Hox* genes provides the basis for anterior-posterior axis specification throughout the animal kingdom (Gilbert 2013).

Lee *et al.* (2003) reported the first *Hox* genes expression patterns in cephalopod mollusk. The results show that *Hox* genes correctly expressed have been repeatedly recruited and in different ways in the origin of the new cephalopod structures. It was demonstrated that the structure of the brachial crown, funnel, stellate ganglia of the mantle and the neurons

well-developed web (depth 1.2 mm) between the two divisions as found uniting the bases of normal arms in this species. The suckers are biserial in the normal fashion from the mouth to immediately below the junction. The second row of

$y = 0.0016x^{2.7175}$
$R^2 = 0.7994$
$n = 115$
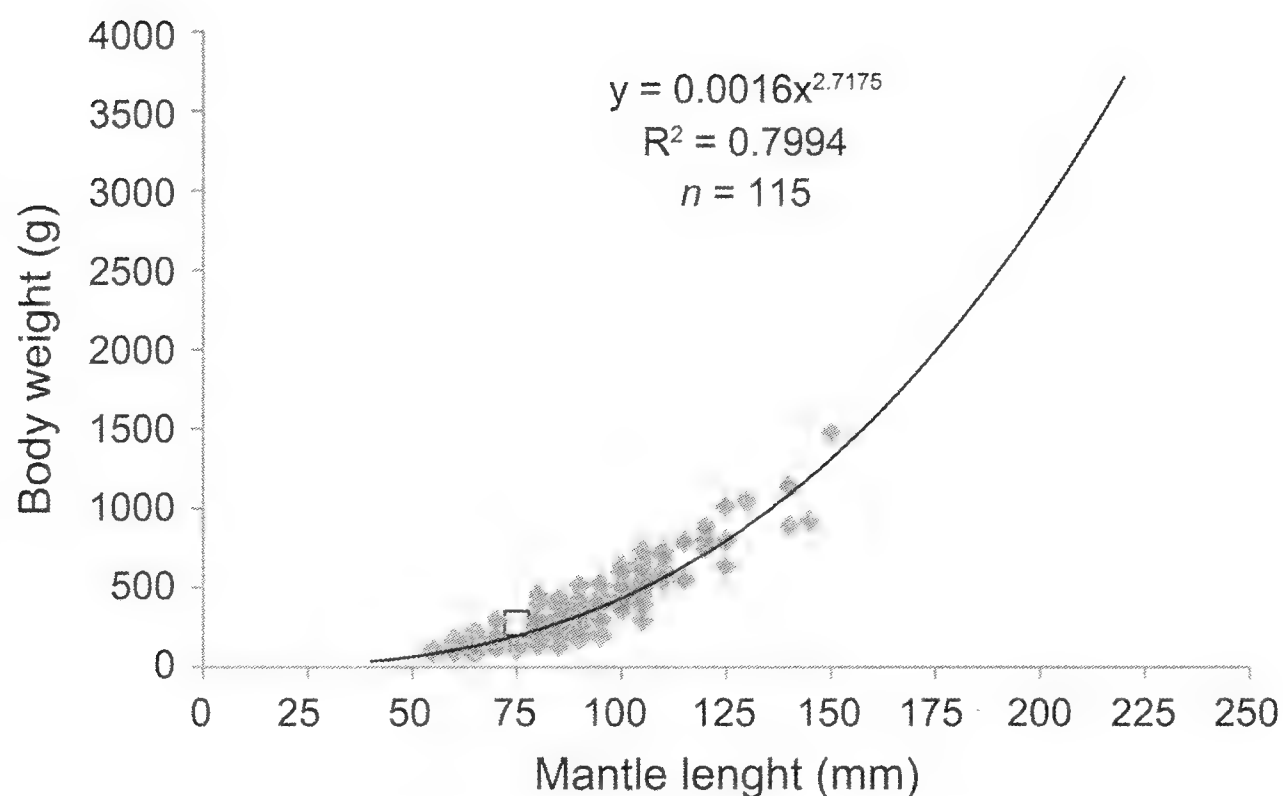
Figure 2. *Octopus hubbsorum* body weight–mantle length relationship. Open square indicates octopus with the bifurcate arm.

that regulate their behavior, including the ganglia and the lobes of the nervous system, are the expression of multiple *Hox* genes interacting coordinately during the embryonic development of that species; each arm pair expresses a unique combination of these genes.

The abnormality observed in *Octopus hubbsorum*, could be due to a *Hox* genes mutation. It was demonstrated that *Hox* genes determine where limbs and other body segments will grow in a developing larva. Also, that mutation in any one of these genes can lead to the growth of extra, typically non-functional, body parts in invertebrates and can produce visible phenotypic changes (Lodish *et al.* 2003).

ACKNOWLEDGMENTS

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RESEARCH NOTE

Octopuses have a fowl diet

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Abstract: There are several references to octopuses eating birds but few give details of the encounters. Here we document the details of seven instances (six *Enteroctopus dofleini* (Wülker, 1910) and one *Octopus* cf. *insularis* Leite and Haimovici, 2008) of octopuses attacking, capturing or eating birds, including glaucous-winged gulls (*Larus glaucescens*), a pigeon guillemot (*Cepphus columba*), a double-crested cormorant (*Phalacrocorax auritus*), a western grebe (*Aechmophorus occidentalis*) a brown noddy (*Anous stolidus*) and a bald eagle (*Haliaeetus leucocephalus*).

Key words: giant Pacific octopus, *Enteroctopus dofleini*, sea birds, octopus predation.

Several stories about octopus-bird interactions are found in the oral traditional literature of the coastal peoples of northeastern Pacific as related by Ellis and Swan (1981) and Eastman and Edwards (1991). Although clearly not in the category of documentary evidence, such information should not be dismissed out of hand. In the cultures where these stories originated, the intelligence and cleverness of the Raven, a supernatural being also called the Trickster, are an understood part of the backstory. In most encounters between the Raven with an octopus, the clever Raven generally wins the day. However, the octopus is also endowed enough intelligence to occasionally best the trickster. The raven may be the most intelligent of the Corvidae, birds known for their intelligence (Seed *et al.* 2009) and the octopus is the most intelligent invertebrate (Mather *et al.* 2010), thus showing the deep understanding of each species possessed by the storytellers.

In some stories, the details are sufficiently clear that they probably indicate a basis in actual and, possibly, quite recent events. Ellis and Swan (1981) document a story of Mr. Raven annoying Miss Octopus as she harvests clams at low tide. He doesn't realize the tide is rising until she grasps him and holds him underwater until he drowns. This rare occurrence when someone gets the upper hand on Raven, attests that octopuses are also clever and, perhaps more importantly from our perspective, that octopuses may occasionally kill (and eat) birds, such as ravens, that frequent the shores at low tide. These stories frequently "have a grain of truth" to them.

A report lists several instances of (presumably) a single octopus that killed two birds that were noted by both experienced octopus observers and birders (Sharpe *et al.* 1991). The documentation implicated a giant Pacific octopus, *Enteroctopus dofleini* (Wülker, 1910), living in a water-filled den under the lower edge of a concrete boat launch ramp that extended down to the lower intertidal zone on Whidbey Island (Washington State). The report noted that observers saw the octopus capture and drown a glaucous-winged gull (*Larus glaucescens*) and a pigeon guillemot (*Cepphus columba*), although it does not record whether the octopus ate the birds. The report further suggests that such man-made constructions on the shore may enhance denning opportunities for *E. dofleini* on the shore and, thus, may give the predatory octopuses more opportunities to catch and eat seabirds and shorebirds.

A recent observation of an *Enteroctopus dofleini* eating a glaucous-winged gull (*Larus glaucescens*) (Fig. 1) was photographed at a breakwater in Victoria, B.C., Canada. The subsequent photographs intrigued the public (see Birdfellow.com, 28 Apr 2012 ff) and provided us with further details of octopuses eating birds.

Ken Wong (KYK Wong Enterprises, Vancouver, B.C.), a professional octopus collector, reported seeing the body of a double-crested cormorant (*Phalacrocorax auritus*) in the midden of an *Enteroctopus dofleini* (K. Wong, pers. comm.) and one of us (RCA) has seen a partially eaten western grebe (*Aechmophorus occidentalis*) in the den midden of an *Enteroctopus dofleini*. The grebe's breast and inner organs had been eaten, leaving the back, wings, neck and head from which remains identification was made. Another professional octopus collector reported seeing "diving ducks" in *E. dofleini* middens (Cliff Law, pers. comm.). In an observation also gleaned from the email list of Birdfellow.com, an encounter between a bald eagle (*Haliaeetus leucocephalus*) and an *E. dofleini* was witnessed where neither could gain the upper hand and both escaped.

An observation of a different species of octopus preying on birds on an island off Brazil was documented by Sazima
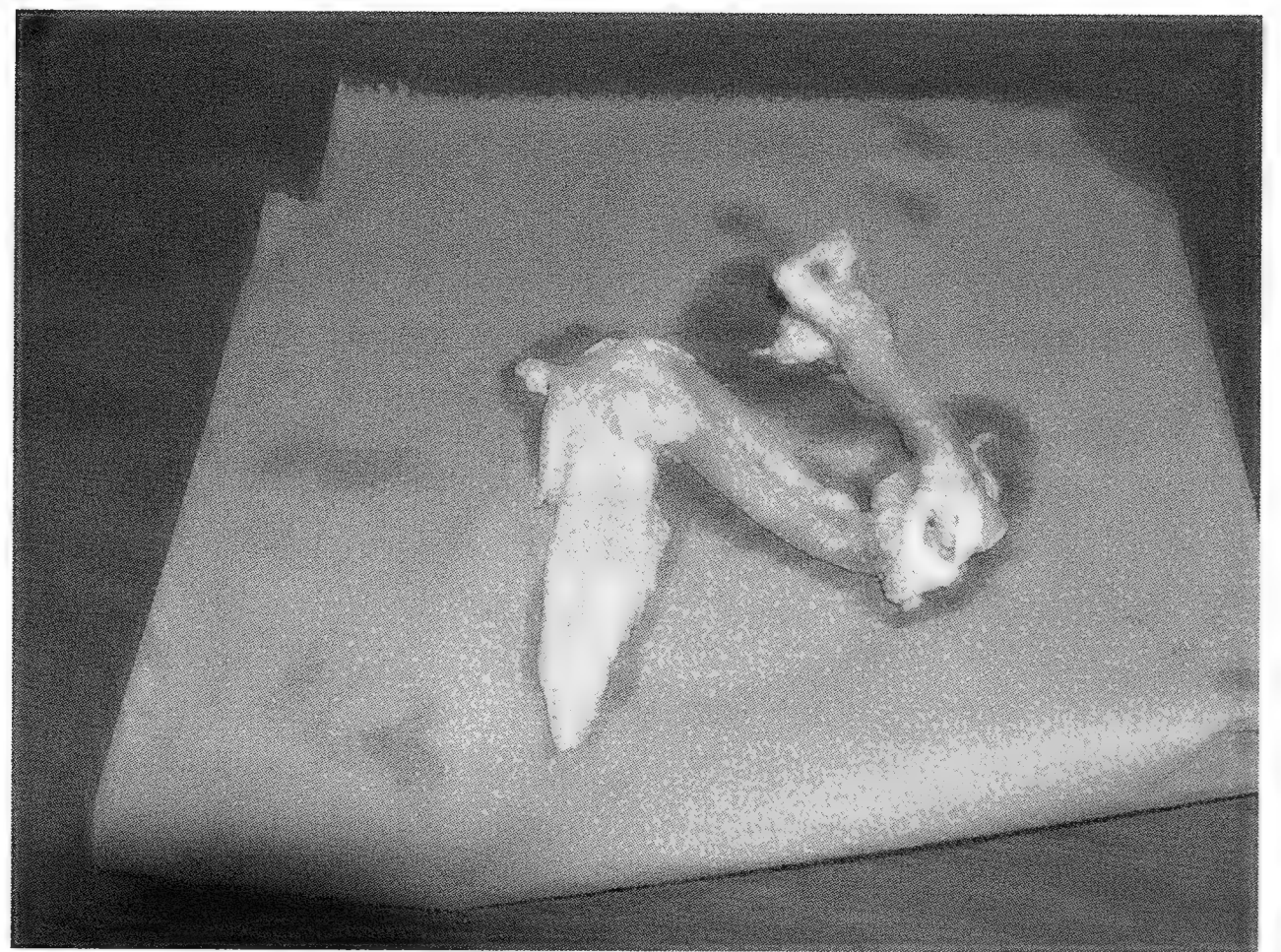
Figure 1. A giant Pacific octopus (*Enteroctopus dofleini*) eats a glaucous-winged gull (*Larus glaucescens*) at a breakwater in Victoria, British Columbia (Canada) while a photographer looks on. Photo by Ginger Morneau.

Figure 2. A raw piece of chicken eaten by a giant Pacific octopus (*Enteroctopus dofleini*) at the Seattle Aquarium (Seattle, Washington, U.S.A.). Photo by Leo Shaw, The Seattle Aquarium.

and de Almeida (2008). They observed an unidentified octopus (*Octopus* cf. *insularis* Leite and Haimovici, 2008) capturing and thence eating a brown noddy (*Anous stolidus*), a gull-like bird that had perched on the edge of a tide pool. The octopus in the tide pool threw two arms encircling the legs of the noddy and held it underwater until it drowned. It then ate the noddy, beginning at the base of the tail (the crissom), working its way up to its neck, holding it for seven hours, when a wave dislodged the remains from the octopus's grip.

It is obvious from these several accounts that birds may make up more than incidental, casual prey of some octopuses. There are an increasing number of these accounts in the literature of multiple species of octopuses eating multiple species of sea birds, shore birds, and diving ducks, and there is no reason not to expect more. It is likely that through further observation by increasing numbers of beach naturalists that the list of birds eaten will expand.

In Alaska, *Enteroctopus dofleini* have been observed under intertidal boulders and the pools surrounding them in south-central Alaska (RLS, pers. obs., Scheel 2002) and to a lesser extent on the west coast of British Columbia and Washington State, thereby increasing the chances of octopuses catching shore birds such as peeps and gulls. And although the observed fight between the bald eagle and the *E. dofleini* ended in a draw, such is probably not always the case. Eagles may forage on the shore at low tide and they can certainly eat small octopuses, but *E. dofleini*, which can grow to more than 40 kg (Mather *et al.* 2010), can certainly grow large enough to pull even an eagle underwater, or to hold it in place as the tide came in.

Anderson (2008) demonstrated the taste captive *Enteroctopus dofleini* have for birds by feeding them raw chicken parts for enrichment purposes (see Fig. 2). Such octopuses were well-fed

in captivity per Anderson (2001) so it is unlikely that octopuses were eating chicken because of extreme hunger. They ate the chicken meat readily and eagerly, and also ate hard-boiled chicken eggs (Anderson 2008) indicating a further taste for fowl.

An octopus's taste is catholic. Anderson *et al.* (2008) reported 75 prey species eaten by *Octopus vulgaris* Cuvier, 1797 in the Caribbean and Scheel and Anderson (2012) report 69 species eaten by *Enteroctopus dofleini*. It may be that octopuses are just well adapted for taking the opportunity to eat anything of the right size that moves although Lee *et al.* (1991) found that chicken-based pelleted food did not support octopus growth in captivity.

It may seem unexpected that octopuses could eat birds with their protective feathers but the suckers of octopuses are well-adapted for prey manipulation (Grasso 2008). Octopuses may be able to bend or wrap their suckers around an individual feather in order to pluck it and thus access birds' flesh underneath the feathers.

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Symposium on the "Magnitude of molluscan diversity – the known and the unknown"

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The symposium formed part of the 78th Annual Meeting of the American Malacological Society in Cherry Hill, New Jersey, and was held on 19th and 20th June 2012. While everyone talks about biodiversity we asked the basic question: What do we know about molluscan diversity? Or even more simply: How many species of molluscs exist today? A simple question to ask but difficult to answer. Of course, this question is not a new one, but ever rising threats to our environment and thus, habitats of molluscs render it more urgent than ever to know what lives on our earth and what will we lose. Land and freshwater molluscs are among the most endangered groups worldwide (Lydeard *et al.* 2004, Régnier *et al.* 2009).

More recent comprehensive estimates of molluscan diversity suggest the number of described species at 31,000 non-marine and 52,500 marine, but the number of yet unknown diversity may be roughly one third to more than an equal amount (Lydeard *et al.* 2004, Bouchet 2006).

The symposium title reflects these two aspects while the "known" refers to described species rather than implies that their number is known considering the often unknown share of synonyms among available names. The goal of the symposium was to gather new data to narrow down a realistic estimate of the diversity of this second largest phylum in the animal kingdom and to reveal the progress made during the last few years.

The 22 talks presented in Cherry Hill encompassed studies on special taxonomic groups, geographic areas as well as theoretical approaches like analyzing saturation curves, the portion of valid species versus synonyms, correlation to rarity, sampling methods, the amount of undescribed species in collections versus discovery by new fieldwork, and last but not least elucidated the potential of new methods. The "unknown" stood for what remains to be discovered in terms of species not yet found in underexplored habitats and regions, species not yet recognized due to the lack of taxonomic expertise, revisionary works, and cryptic species.

If the title was mirrored in two talks, Gary Rosenberg's analyses of databases reducing the estimated diversity of described species to 70,000–76,000 represented the "known" (this issue) while Philippe Bouchet certainly took the audience to the frontier of the "unknown". Based on his tremendous exploration efforts in the tropical Indo-Pacific Bouchet assumed that only 30% of the molluscan diversity of this area is known. This already anticipates that despite some detailed approaches the latter aspect remains not only as undescribed diversity but still unknown in its extent.

In terms of marine species, Rüdiger Bieler questioned our knowledge of the molluscan fauna in the well known Florida Keys. Philippe Bouchet, as mentioned above, characterized the tropical Indo-Pacific as a treasure trove for new species and highlighted the phenomenon of a high percentage of species occurring in low abundances. Janet Voight evaluated the exploration of hydrothermal vent habitats (this issue). John Taylor, co-authored by Emily Glover, gave insight into the enormous diversification of Lucinidae triggered by their obligate chemosymbiosis.

Continuing with bivalves, Paul Valentich-Scott dug deep into the hyperdiverse group of Galeommatoidea which not only amaze us with their previously unexpected species diversity but even more by their range of unusual lifestyles. Patrick LaFollette described the attempt to catalogue the published taxa of the megadiverse family Pyramidellidae resulting in an estimated 10,000 names regardless of status. James McLean's review of the Liotiidae and Areneidae more than doubles the known diversity and sampling still seems far from complete. Exposing the most cryptic groups, Timea Neusser and colleagues gave insights into the mesopsammic fauna and their underestimated diversity and ability to colonize unusual habitats (this issue, Jörger *et al.*). Moving from the least known to the best known, Fabio Moretzsohn reviewed the progress of cataloging the Cypraeidae (this issue). An equally attractive group but less popular among shell collectors, Terrence Gosliner described nudibranch exploration. Greatly supported by citizen scientists like scuba divers, Gosliner feels their role is not only in accelerating the discovery of new species but also in helping to monitor environmental changes.

The contributions focusing on advances by the application of molecular techniques identified significant numbers of so-called cryptic species: Ellen Strong estimated the diversity of Cerithiidae two to three times higher than currently recognized. John Slapcinsky deduced equal ranges from his analyses of the land snail genera *Tropidophora* and *Daedolochila* (this issue, on *Tropidophora*). To the relief of less well-equipped biologists both pointed out that after recognizing the hidden species differentiating morphological characters

Figure 1. Speakers at the Symposium "*Magnitude of molluscan diversity – the known and the unknown.*" Front row, left to right: Philippe Bouchet, Adrienne Jochum, Timea P. Neusser, Ira Richling, John Slapcinsky, Arthur E. Bogan, John D. Taylor, and Fabio Moretzsohn. Second row, left to right: Eike Neubert, Janet R. Voight, Ellen E. Strong, Jeffrey C. Nekola, Rüdiger Bieler, Jan Johan ter Poorten, and Terrence Gosliner. Back row, left to right: Gary Rosenberg and Philip J. Fallon, Jr. Missing from photo: Frank Köhler, Patrick I. LaFollette, James H. McLean, Paul Valentich-Scott, and Francisco Welter-Schultes. Photo by Klaus Groh.

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were usually found as well. In contrast, Adrienne Jochum, co-authored by Alexander Weigand, favored molecular identification for Carychiidae to deal with high intraspecific morphological plasticity in combination with a general conchological uniformity among taxa (this issue, Weigand *et al.*).

Regarding terrestrial diversity Jeffrey Nekola discussed the land snails of North America north of Mexico (this issue), Eike Neubert summarized the current knowledge for the continental molluscs of the Western Palaearctic. Frank Köhler provided an overview for Australia and estimated another 67% of still undescribed species. Ira Richling gave a critical estimate for the family Helicinidae as example for mainly forest dwelling tropical land snails that are faced with dramatic habitat loss (this issue). Arthur Bogan discussed freshwater molluscan diversity worldwide and elaborated on the topic of vanishing diversity.

Besides the scientific questions, Jan Johan ter Poorten (on Cardiidae) and Philip Fallon (on Drillidae) presented a perspective from the amateur's side, citizen scientists who provide the main taxonomic workforce in describing new species. Finally, Francisco Welter-Schultes brought it back to the practical basics with an overview of available databases and advantages versus shortcomings of the different nomenclators compiled over time with the insider view of a provider of such data.

I thank Gary Rosenberg for the invitation to organize the symposium along with Philippe Bouchet who took the lead for

Overview of the North American terrestrial gastropod fauna*

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Abstract: A revised database of terrestrial gastropods from North America north of Mexico was assembled in the spring of 2012, which included not only all likely species-level entities, updated family and naturalized exotic assignments, but also shell and body size data. Analyses of these reveal that: (1) the fauna represents approximately 1,200 species, and is dominated by the Polygyridae, Helminthoglyptidae, and Vertiginidae. This number is surprisingly small, with other land masses of ½ to 1/100th the size possessing a larger fauna; (2) naturalized species make up 6% of the total fauna; (3) while slugs represent only 7% of the native fauna, they constitute over 1/3 of the naturalized fauna; (4) the accumulation curve for recognized species is sigmoidal, with current rates being the lowest experienced in 200 years. As a result, it appears that only 150–300 net additional taxa may await description, with the principle future taxonomic activity being revisionary work; (5) like other faunas, both native and naturalized land snails in North America possess bimodal height/width ratios; (6) native land snails possess a bimodal, right-skewed biovolume distribution along a log axis, which differs greatly from the unimodal left-skewed distribution typical of many other taxa groups; and, (7) the distribution of species within families and genera, and of the number of species described by a given researcher all possess Power Law/Log Normal distributions characteristic of complex adaptive systems.

Key words: Body size patterns, complex systems, diversity, land snail, macroecology

With the publication of the last volume of the "Land Mollusca of North America (North of Mexico)" by Henry Pilsbry in 1948, this fauna became the first to be taxonomically well-described at the continental level. However, since this time efforts to maintain and update his work have been principally limited to Leslie Hubricht's county-scale range maps for eastern U.S.A. species (Hubricht 1985) and two checklists of common and scientific names for the Mollusca of the United States and Canada published by the American Fisheries Society (Turgeon *et al.* 1988, 1998). While representing a massive undertaking, the latter include only very transcribed ecological information (limited to native or alien status), use a now outdated family-level taxonomy, and do not consider any taxa listed by Pilsbry (1948) or subsequent workers as subspecies. Because Hubricht (1985) tended to split these entities into separate species, this is less of an issue for the eastern U.S.A. fauna. However, since little revisionary work has been conducted on the western U.S.A. fauna, a strong potential exists for this policy to lead to underreporting of valid species in this region. And, obviously, no species described in the almost 15 years since publication of the second edition of Turgeon *et al.* were included.

To address these concerns, an updated list of the North American terrestrial gastropod fauna north of Mexico was assembled during the spring of 2012. It not only updates family level taxonomy to current concepts, but also includes all likely species that Pilsbry (1948) lists as subspecies, and includes all species described through May 2012. This dataset also records median shell/body size dimensions from the published literature and from this calculates shell biovolume for each entry. As a result, a current and modern overview of this terrestrial gastropod fauna can be constructed.

MATERIALS AND METHODS

Faunistic enumeration

Only species having native or naturalized populations in North America north of Mexico were considered; species occurring south of the U.S.A. boundary are considered part of the Central American fauna for purposes of this work. While an initial listing of the Central American fauna is now available (Thompson 2011), these taxa have not been included because of: (1) the historical limitation of previous works (*e.g.,* Pilsbry 1948, Turgeon *et al.* 1998) to North America north of Mexico; (2) the much poorer understanding of alpha-level taxonomy in the Central American fauna; and (3) their often distinct evolutionary affinities. Although a number of North American

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elements extend south into at least northern Mexico (*e.g.*, the genus *Ashmunella* Pilsbry and Cockerell, 1899 and the subgenus *Immersidens* Pilsbry and Vanatta, 1900 within the genus *Gastrocopta* Wollaston, 1878), and some Caribbean and Central American elements extend north into the southern U.S.A. (*e.g.*, the Urocoptidae and Orthalicidae), the artificial demarcation used here is similar to those erected by molluscan biogeographers elsewhere, such as in Eurasia where European, Asian, east Asian, and south Asian faunas are usually considered as largely separate entities (Naggs and Raheem 2005, Pokryszko and Cameron 2005).

The initial point of departure was all terrestrial gastropod entries from Turgeon *et al.* (1998). To this were added all subsequently described species as determined via the *Zoological Record* database. Additionally, all species listed by Hubricht (1985) – but not by Turgeon *et al.* (1998) – were included. Most of these represent dead shells limited to drift along the south Texas Gulf Coast. While their presence in North America may be due to long-distance rafting from extralimital colonies (the apparent rationale used to justify exclusion), it seems equally likely that they were sourced from nearby, localized extant colonies. Inclusion of these taxa, thus, seems prudent to ensure that no extant species were ignored. The list was also expanded to include all subspecies-level entities from Pilsbry (1948) that appear, based on their unique shell features, ranges, and/or ecological preferences, to represent valid species-level entities. Pilsbry (1948) took a conservative approach to species designations, and chose to lump numerous forms as subspecies until appropriate data existed to falsify these hypotheses. As a result, many species-level entities were listed by Pilsbry (1948) as subspecies, as has been recently shown within the Polygyridae (Emberton 1988), Helminthoglyptidae (Lang and Gilbertson 2010), and Vertiginidae (Nekola *et al.* 2009). However, not all of Pilsbry's subspecies concepts appear to warrant species-level listing: examples are the minor shell variants limited to specific canyons within a given species range, as demonstrated not only by subspecies described within *Ashmunella proxima* Pilsbry, 1905 and *A. levettei* (Bland, 1881) of the Chiricahua and Huachuca ranges of southeastern Arizona, respectively, but also in various species within the genus *Oreohelix* Pilsbry, 1904.

Finally, fourteen undescribed new species encountered by the author during field sampling across North America have also been included. These represent six *Vertigo* Müller, 1774, plus single representatives of *Columella* Westerlund, 1878, *Daedalochila* Beck, 1837, *Glyphyalinia* von Martens, 1892, *Hawaiia* Gude, 1911, *Helicodiscus* Morse, 1864, *Paravitrea* Pilsbry, 1898, *Punctum* Morse, 1864, and *Succinea* Draparnaud, 1801.

Taxonomic information

The authorship of each entry was recorded, as was the year of description. Family assignments were updated to Bouchet and Rocroi (2005), with the placement of genera into these families basically following Schileyko's 1998–2006 "*Treatise on Recent Terrestrial Pulmonate Molluscs*" as used in the Academy of Natural Sciences of Drexel University (ANSP) collection.

Ecological information

Species native or naturalized status was based largely on Turgeon *et al.* (1998) with subsequent additions and changes based on Nekola and Coles (2010). Species with documented pre-Columbian occurrences in North America are treated as native even though some (*e.g.*, *Cepaea hortensis* Müller, 1774) may represent earlier European colonizations via the Norse or Basque. Each entry was assigned either as a slug or snail, with semislugs being classed as snails due their presence of a relatively large external shell. Body dimensions for each entry was determined from either the published literature, or in the case of undescribed taxa from lots held in the Nekola collection. For snails, median reported shell height (mm), width (mm) and proportion of shell height which is columnar were recorded. Shell biovolume in mm^3 was then estimated as a conic-section placed on top of a column (McClain and Nekola 2008) using the formula:

$$V = \pi r^2 ht + \left[\frac{\pi r^2 h(1-t)}{3} \right]$$

where: V = biovolume in cubic mm; h = height; r = width/2; t = taper proportion varying from 1 = completely columnar shell height to 0 = completely tapered shell height.

For slugs, the median width and diameter in mm was recorded for relaxed individuals. Slug biovolume in cubic mm was simply estimated as a cylindrical column:

$$V = \pi r^2 h$$

Analyses

Richness at the species, genus, and family level were calculated for the native and naturalized fauna. The largest families and genera in terms of species richness were also determined. The accumulation of currently accepted names over time was plotted for the native fauna, with the mean number of described entities calculated for five different chronological eras (1774–1815, 1816–1889, 1890–1942, 1943–1990, and 1991–present) whose boundaries were based upon rate discontinuities in the accumulation graph. The number of species described per author was also determined within the native fauna. Two general analyses were conducted on the biovolume data: First, the body-size spectrum of the native and naturalized faunas were displayed for snails and slugs via histograms based on 41 log$_2$ volume classes with bins starting at -2.5 and increasing by 0.5 log$_2$ units to 18.0. The first bin value ranged from -2.5 to -2.0 (~0.18–0.25 mm^3) and

the last bin from 17.5–18.0 (185,363.8–262,144 mm³). The number of species were tabulated within each log₂ volume bin and plotted. Second, a histogram of natural log-transformed height-width ratios was generated for both the native and naturalized land snail faunas. Log transformation was used along the height-width axis as it allowed for a similar spread in values for shells less than or greater than 1.0. Without this transform, the range of variation for shells wider than tall would be compressed into ¼ of the range covered by shells that were taller than wide, complicating analysis.

RESULTS

Fauna size and composition

A total of 1,204 terrestrial gastropod species level entities were recorded (Table 1; Appendix 1), representing 170 genera and 51 families; 1,128 species, 136 genera, and 41 families are native to the continent, or at least of pre-Columbian occurrence. Seventy six of these had been previously recorded as subspecies by Pilsbry (1948). Across the native fauna, 84 species, 28 genera, and eight families represent slugs. The naturalized fauna represents 76 species, 46 genera, and 26 families, of which 26 species, 12 genera, and six families are slugs.

By far the most diverse family represented in the native fauna (Table 2) is the Polygyridae with 271 species (24.0% of native fauna), followed by the Helminthoglyptidae (178; 15.8%), Vertiginidae (102; 9.0%), Oreohelicidae (84; 7.4%), and Pristilomatidae (65; 5.8%). The ten richest families contain a total of 884 likely species-level entities, or 78.4% of the total fauna. On the other side of the distribution, one family (Cochlicopidae) is represented by only three species, four families (Charopidae, Pomatiidae, Pomatiopsidae, and Vitrinidae) by only two species, and six families (Cepolidae, Ferrussaciidae, Helicidae, Oleacinidae, Sagdidae, and Veronicellidae) by only a single native species. The most diverse genus in the fauna is *Oreohelix* (79 species, or 7.0% of the native fauna) followed by *Sonorella* Pilsbry, 1900 (71; 6.3%), *Helminthoglypta* Ancey, 1887 (70; 6.2%), *Vertigo* (65;

Table 2. Most diverse families and genera at the species level.

A. Native fauna

Family	Species	Genera	Species
Polygyridae	271	*Oreohelix*	79
Helminthoglyptidae	178	*Sonorella*	71
Vertiginidae	102	*Helminthoglypta*	70
Oreohelicidae	84	*Vertigo*	65
Pristilomatidae	64	*Ashmunella*	53
Succineidae	48	*Paravitrea*	42
Oxychilidae	45	*Daedalochila*	34
Gastrodontidae	35	*Stenotrema* Rafinesque, 1819	29
Urocoptidae	31	*Triodopsis* Rafinesque, 1819	28
Discidae	26	*Gastrocopta*	27

B. Naturalized Introduced fauna:

Family	Species	Genera	Species
Arionidae	10	*Arion*	10
Subulinidae	8	*Lamellaxis*	4
Hygromiidae	8	*Limax*	4
Helicidae	7	*Oxychilus*	4
Veronicellidae	7		
Limacidae	5		
Oxychilidae	4		
Pleurodontidae	4		

5.8%), and *Ashmunella* (53; 4.7%). The ten most diverse genera contain 498 species, or 44.1% of the total fauna. On the other side of the distribution, eleven genera are represented by only three species, 17 genera by only two species, and 45 genera by on a single one native species.

Eight families (Arionidae, Helicidae, Hygromiidae, Limacidae, Oxychilidae, Pleurodontidae, Subulinidae, and Veronicellidae) constitute almost three-quarters of the post-Columbian naturalized fauna (Table 2B). On the other end of the distribution, one family is represented by only three species, three families by only two species, and fourteen families by only a single naturalized species. By far and away *Arion* Férussac, 1819 represents the most diverse genus of naturalized species (10 species, or 14.1% of the naturalized fauna), followed equally by *Lamellaxis* Strebel and Pfeffer, 1882, *Limax* Linné, 1758, and *Oxychilus* Fitzinger, 1833 (4; or 5.6% each). Two genera are represented by only three species, eight genera by only two species, and 32 genera by only a single naturalized species.

Alpha-level taxonomy history

Since the first description of a native North American terrestrial gastropod species by Danish naturalist Otto Friedrich Müller in 1774, a total of 149 investigators have

Table 1. Overview of North American terrestrial gastropod fauna.

	Species	Genera	Families
Total	1204	170	51
(slugs)	84	28	8
Native	1128	136	41
(slugs)	58	18	6
Introduced	76	46	26
(slugs)	26	12	6

contributed to the naming of the native fauna. The top ten contributors include (Table 3): Henry A. Pilsbry, James H. Ferriss, Leslie Hubricht, Thomas Say, S. Stillman Berry, Victor Sterki, Walter B. Miller, Thomas B. Bland, Augustus A. Gould, and William G. Binney. On the other side of this distribution fourteen individuals have contributed to the description of only three species, twenty to only two species , and 61 to only a single native species.

The accumulation of recognized native North American taxonomic descriptions over time exhibits a sigmoidal pattern (Fig. 1). Initial description rates were low (0.2 species / year), and restricted to charismatic or Holarctic species described by European researchers. Beginning with Thomas Say in 1816, North American malacologists began in earnest the naming of their fauna, describing through the 1880s and average of 4.4 species a year. The most productive era began in 1890 with the start of Pilsbry's career at ANSP: through 1942 an average of 10.3 new North American terrestrial gastropod species would be described per year. These rates decreased to an average of 4.6 per year from 1943–1990, and have decreased further to an average of 1.8 per year for the last decade of the 20th Century through the first dozen years of the new millennium.

Body Size and Shell Shape Patterns

Native North American land snails (Table 4a) range in size from the 1.1 mm x 0.6 mm *Punctum minutissimum* (I. Lea, 1841) to the 34 mm x 61 mm *Orthalicus floridensis* Pilsbry, 1889. Native slugs range in size from the 4.0 mm x 2.0 mm *Deroceras leave* (Müller, 1774) to the 222 mm x 37 mm *Ariolimax columbianus* (Gould, 1851). While 264 native species

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Table 3. Most prolific describers of the North American terrestrial gastropod fauna.

Individual	Species Described	Inclusive Years	Emphasis
Henry A. Pilsbry	305	1889–1953	Whole continent
James H. Ferriss	82	1900–1925	SW U.S.A.
Leslie Hubricht	79	1938–1983	E U.S.A.
Thomas Say	51	1816–1831	E. North America
S. Stillman Berry	46	1916–1955	California
Victor Sterki	35	1889–1919	Whole continent (pupillids)
Walter B. Miller	31	1966–2000	W U.S.A. (polygyrids and helminthoglyptids)
Thomas B. Bland	28	1856–1883	S and W U.S.A.
Augustus A. Gould	26	1840–1855	Whole continent
William G. Binney	26	1857–1892	Whole continent

(24.7% of total) have a maximum dimension < 5 mm, 201 (18.8%) range from 5 ≤ 10 mm, 403 (37.7%) from 10 ≤ 20 mm, 197 (18.4%) from 20 ≤ 40 mm, and 5 (0.5%) are ≥ 40 mm. One native slug (1.7% of all native slugs) has a maximum dimension < 5 mm, while five (8.6%) range from 5 ≤ 10 mm, 15 (25.9%) from 10 ≤ 20 mm, 18 (31.0%) from 20 ≤ 40 mm, and 19 (32.8%) are ≥ 40 mm.

Naturalized land snails (Table 4b) range in size from the 0.9 mm x 1.8 mm *Carychium minimum* Müller, 1774 to the 36 mm x 35 mm *Helix pomatia* Linnaeus, 1758. Naturalized exotic slugs range in size from the 20.0 mm x 3.3 mm *Arion intermedius* Normand, 1852 to the 150 mm x 25 mm *Limax maximus* Linnaeus, 1758. While eleven naturalized land snails (22.07% of total) have a maximum dimension < 5 mm, thirteen (26.0%) range from 5 ≤ 10 mm, fourteen (28.0%) from 10 ≤ 20 mm, and twelve (24.0%) from 20 ≤ 40 mm. No naturalized North American snail equals or exceeds 40 mm in maximum dimension. No naturalized exotic slug has maximum dimensions < 20mm. Eight naturalized slugs (30.8% of all naturalized slugs) have a maximum dimension from 20 ≤ 40 mm while eighteen (69.2%) are ≥ 40 mm.

Biovolume represents a more accurate expression of body size because it considers both height and width. Native North American land snails range from the 0.18 mm^3 *Carychium nannodes* G. H. Clapp, 1905 to the 33,230 mm^3 *Orthalicus floridensis*. The body size spectrum (Fig. 2a) exhibits a bimodal distribution with one mode ranging from 1.4–2.0 mm^3, and the other from 512–724 mm^3. The minimum between these two modes occurs from 16–22.6 mm^3. The right mode exhibits a clear left-skew, although the left mode appears approximately log-normal. Native slugs range from the 7.9 mm^3 *Udosarx lyrata* Webb, 1959 to the ~240,000 mm^3 *Ariolimax columbianus*, with the mode occurring at 362–512 mm^3. The biovolume distribution of these species was approximately log-normal, but shifted slightly to the right of land snails.

Naturalized land snails range from the 0.51 mm^3 *Carychium tridentatum* (Risso, 1826) to the 23,091 mm^3 *Helix pomatia*. The body size spectrum (Fig. 2b) exhibits essentially a uniform distribution across this range. Naturalized exotic slugs range from the 171 mm^3 *Arion intermedius* to the ~74,000 mm^3 *Limax maximus*, with the mode occurring at 362–512 mm^3. The biovolume distribution of these species was approximately log-normal, but shifted to the right of land snails.

Lastly, the height vs. width ratio for both native and naturalized North American land snails is also strongly bimodal (Fig. 3) with the left mode ranging from 0.50–0.67 (1:2–2:3) in both and from 1.8–2.2 (9:5–11:5) in the native and from 3.3–4.1 (10:3–4:1) in the naturalized faunas. Almost no native or naturalized species possess shell height vs. width ratios ranging from 1.0–1.5 (1:1–3:2).

Figure 1: Accumulation curve for recognized native North American terrestrial gastropod species over time.

the continent, with regional values ranging from 56% of species and 60% of individuals on an average Alaskan site to 17% of species and 21% of individuals on an average Florida site (Nekola, *unpub. data*). The next most important family at the site scale (the Gastrodontidae) possesses values approximately three times smaller. Thus, whether the North American terrestrial gastropod fauna should be characterized as being dominated by polygyrids, helminthoglyptids or vertiginids is squarely dependent upon the observational scale: while the former two are most important at the regional to continental scale, on individual sites the vertiginids dominate.

Second, the overall proportion of exotic species is relatively low, representing only approximately 6% of the total fauna. However, this level can locally range much higher, especially at low latitudes. Many disturbed sites in south Florida, for instance, are dominated by a host of naturalized exotics in the Bradybaenidae, Helicarionidae, Orthalicidae, Pleurodontidae, Streptaxidae, and Subulinidae. Helicids and hygromiids may also be locally important components of disturbed faunas in both the northeast and southwest. The importance of naturalized exotics is especially notable in the slug fauna: while slugs represent only 7% of the entire native fauna, they represent 1/3 of the introduced fauna, with fully ten species within the genus *Arion* having become established on the continent. It also seems likely that additional naturalized species may exist within the fauna, even though historically having been considered native. For instance, *Pupilla muscorum* Linnaeus, 1758, is represented in North America by naturalized European populations which are restricted to the urban corridor of the Northeast west through the Great Lakes. Individuals occurring along roadside verges in Cedar Rapids, Iowa have identical mitochondrial and nuclear haplotypes to material originating from Brno in the Czech Republic (Nekola, *unpub. data*). Northern and western populations of *Pupilla 'muscorum'*, however, represent native taxa that are not closely related to this species (Nekola *et al.* in review). It seems likely that similar mixes of native and introduced populations will be uncovered in other anthropophilic Holarctic 'species', such

DISCUSSION

It is a testament to Pilsbry that over 60 years after publication of his North American monograph, in broad brushstrokes his summary of the terrestrial gastropod fauna remains largely unchanged: the fauna includes approximately 1,200 species, with the Polygyridae and Helminthoglyptidae dominating. Yet, there are a number of important additional insights which can be drawn:

Faunistic summary

First, an often overlooked but crucial component of the fauna are the vertiginids, which constitute the third most diverse family, with the genera *Vertigo* and *Gastrocopta* being the fourth and tenth most-speciose genera, respectively. Native vertiginids encompass approximately 1/3 of the total global richness for the entire family (Liggia 2012) and at least 2/3 of global richness for the genus *Vertigo* (Nekola *et al.* 2009), making North America a globally important biodiversity center for the group. At the site scale, the importance of this family is even greater: vertiginids represent roughly 1/3 of the species and individuals found on an average site across

Table 4. Body size distribution across the North American fauna.

A. Native Species

Maximum Dimension	Minute (< 5 mm)	Small (5–10 mm)	Medium (10–20 mm)	Large (20–40 mm)	Very Large (40+ mm)
Land Snails					
Number of taxa	264	201	403	197	5
Percent	24.7	18.8	37.7	18.4	0.5
Slugs					
Number of taxa	1	5	15	18	19
Percent	1.7	8.6	25.9	31.0	32.8

B. Naturalized Species

Maximum Dimension	Minute (< 5 mm)	Small (5–10 mm)	Medium (10–20 mm)	Large (20–40 mm)	Very Large (40+ mm)
Land Snails					
Number of taxa	11	13	14	12	0
Percent	22.0	26.0	28.0	24.0	0
Slugs					
Number of taxa	0	0	0	8	18
Percent	0	0	0	30.8	69.2

as *Cochlicopa lubrica* (Müller, 1774), *Cochlicopa lubricella* (Porro, 1838), *Deroceras laeve* (Müller, 1774), *Vallonia costata* (Müller, 1774), *Vallonia excentrica* Sterki, 1893, and *Vallonia pulchella* (Müller, 1774).

Third, even though the North America terrestrial gastropod fauna supports roughly twice the number of species as better known invertebrate groups such as butterflies and skippers (Scott 1986), it is surprisingly depauperate as compared to other terrestrial gastropod faunas. Of the estimated 35,000 total global terrestrial gastropod species (Barker 2001), approximately 3,600 occur in Europe (Bank *et al.* 1998), more than 2,500 in Australia (John Stanisic, *pers. comm.*), and 1,400 in New Zealand (Barker and Mayhill 1999). Thus, North America supports fewer species than land masses ranging from one-half to 1/100th of its size.

Last, it is important to emphasize that these patterns are subject to change as additional data becomes available. For instance, in the time between initial drafting and editorial acceptance of this manuscript, preliminary results (Nekola, *unpub. data*) revealed that considerable revision of higher taxonomic concepts will likely be necessary within the Pupillidae and Vertiginidae. In particular, the Vertiginidae do not appear to be a distinct family, with most members being distributed throughout the Pupillidae. And, other families, such as the Valloniidae, also appear to represent pupillids. As a result, the importance of the Pupillidae within the North American fauna is likely even greater than indicated above. Additionally, some putative vertiginids—such as the

genus *Columella*—are actually chondrinids. And, it is also clear that the genus *Vertigo* itself has been badly oversplit and actually includes other genera such as *Sterkia* Pilsbry, 1898. These data also strongly suggest that *Vertigo* AK2 and *Vertigo* AK4 represent populations of the Eurasian *V. ronnebyensis* Westerlund, 1871 and *V. microspharea* Schileyko, 1984, respectively. However, until such preliminary findings have been vetted through the review process, they must be ignored within the context of this work.

Taxonomic activities

These analyses document that the shape of the recognized species vs. description year curve is sigmoidal, with current rates for new species descriptions being the lowest seen in almost 200 years. It is important to note, however, that the asymptotic number of recognized species is only being approached but has not yet been met. As a result it seems likely that there are another 150-300 additional net species in the fauna awaiting description. These taxa, however, will likely be named at ever decreasing rates.

The taxonomic activity that will take on increasing precedence is, thus, revisionary work, in which the true status of the various named entities are subjected to empirical validation. Because of their relatively easy mutability, conchological features alone may not be adequate to demarcate species-level groups. For instance, the variable presence of an angular and basal lamella, of the length and degree of inset of the upper palatal lamella, depth of depression over the palatal lamellae,
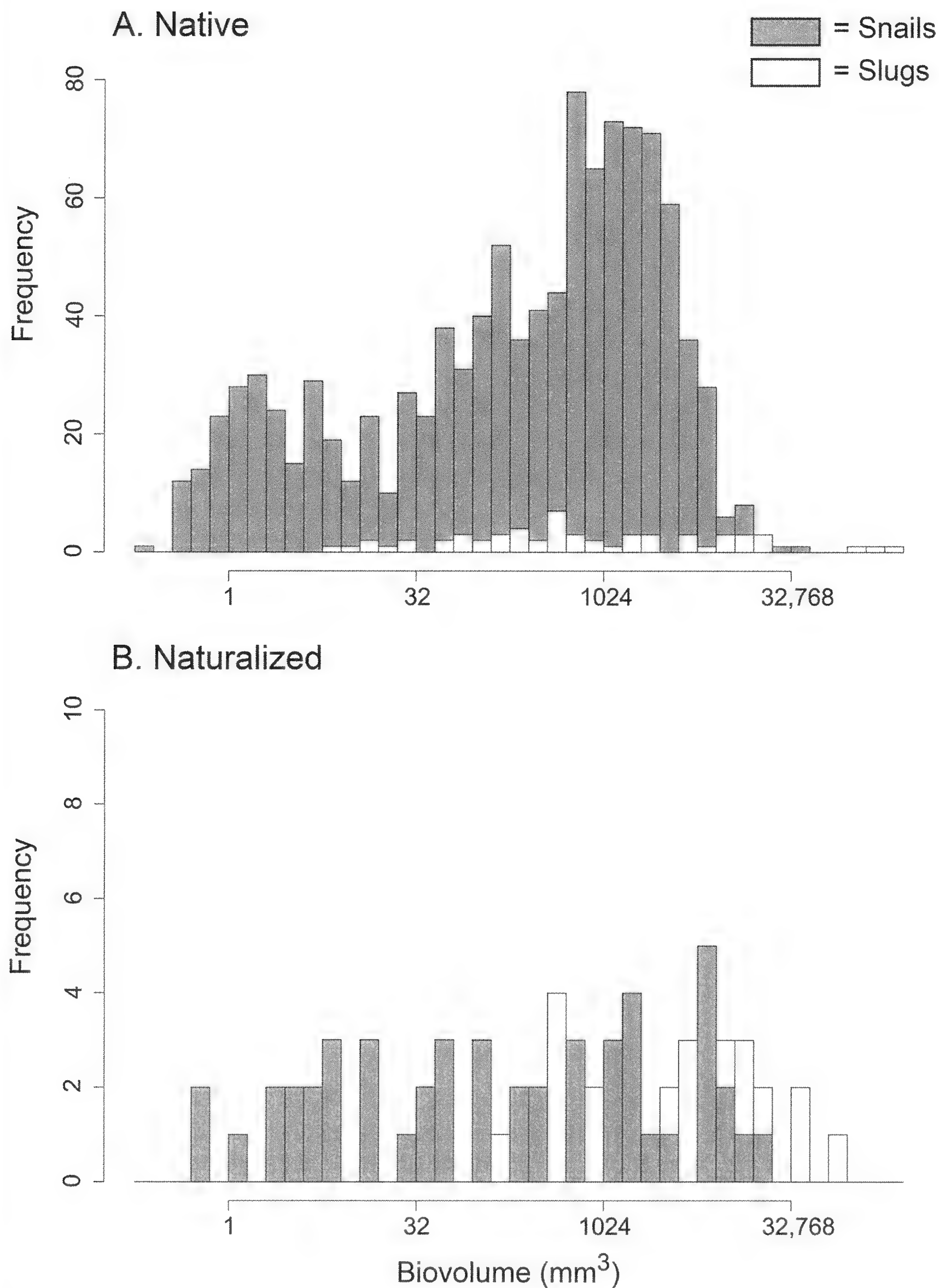
Figure 2: Body size spectra histograms for native and naturalized North American terrestrial gastropods along a $\log_2$ axis, using 0.5 unit bin widths. Filled boxes represent the frequency of snails, while the open boxes represent the frequency of slugs.

and strength of an apertural crest and callus in *Vertigo arthuri* von Martens, 1884 led to the splitting of this entity into six separate taxa (*Vertigo paradoxa* (Sterki, 1900), *V. gouldii basidens* Pilsbry and Vanatta, 1900, *V. hubrichti* (Pilsbry, 1934), *V. brierensis* Leonard, 1972, *V. occulta* Leonard, 1972, *V. iowaensis* Frest, 1991; Pilsbry 1948, Frest 1991), even though they display almost no genetic divergence (Nekola *et al.* 2009). Current analyses on *Pupilla* Leach in Fleming, 1828 across Europe, central Asia, Japan, and North America also indicates

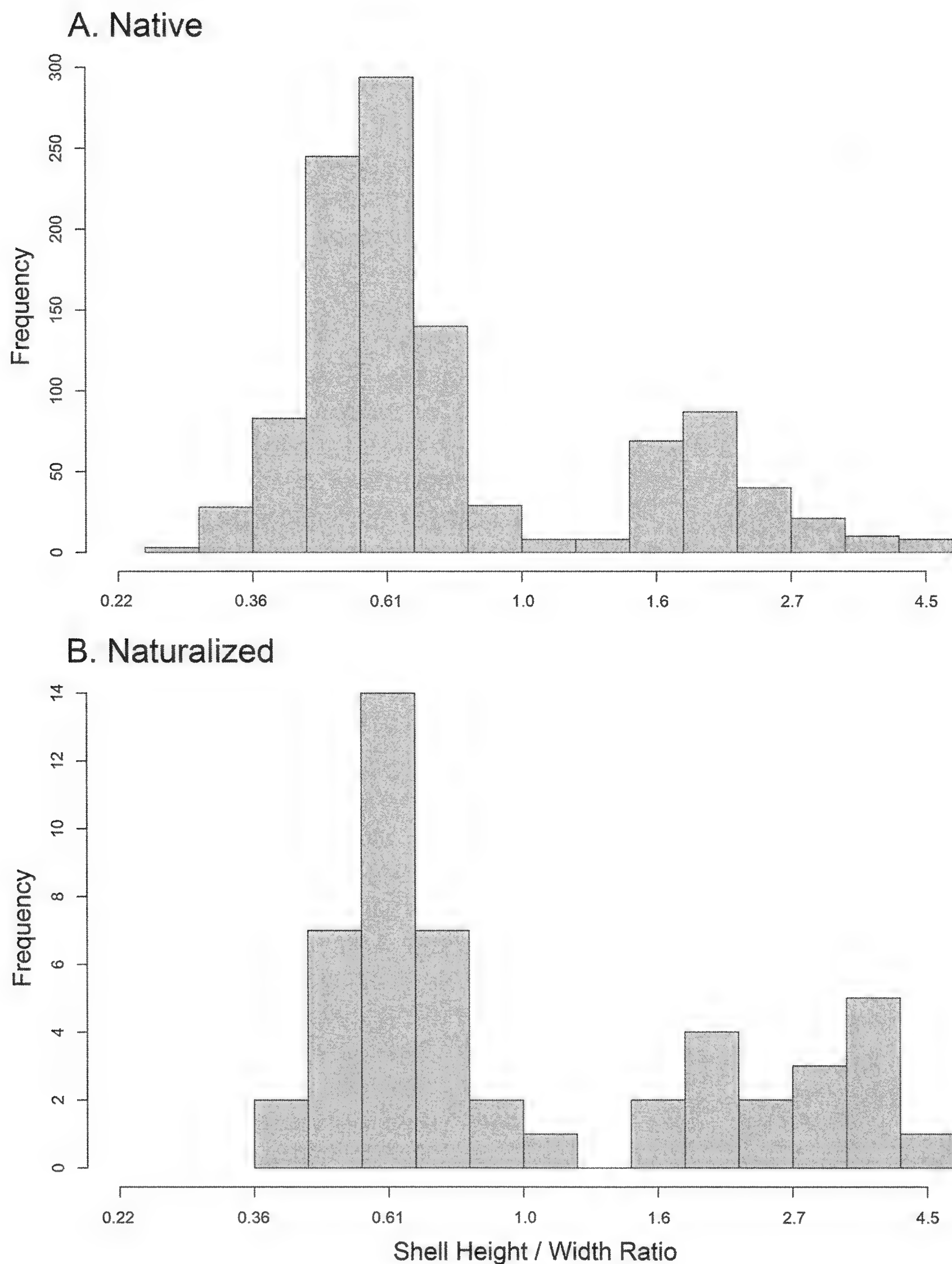profound plasticity in some shell features, rendering invalid many historical species concepts (Nekola *et al.*, in review). However, even in extreme cases such as these, stable conchological traits usually exist to reliably demarcate genetically-distinct species level clades, although these may not be the features that have been historically considered.

Sole use of anatomical features may also lead to faulty conclusions about species-level distinctions. For instance, radular teeth number and configuration are potentially highly unreliable indicators of taxonomic relatedness because they can change with ontogeny (*e.g.*, Bertsch 1976) and are also often under strong ecological selection pressure (*e.g.*, Kesler *et al.* 1986). Also, because gastropods do not exhibit a hard exoskeleton, there is no reason to expect *a priori* that they will possess Arthropod-style lock-and-key genitalia. Rather, gene transfer is at least theoretically possible between individuals with varying genitalic morphologies. DNA sequence analysis demonstrates that at least some anatomically-described North American species are only simple forms within a single, larger variable species (Roth *et al.* 2013). Considerable ontogenetic variability also occurs in many genitalic features (Emberton 1985), with individuals within a single *Catinella* Pease, 1870 population passing through multiple described anatomical 'species' over the course of ontogeny (Brian Coles, *pers. comm.*). Little hard data exists regarding how much genitalic variability exists within and between populations of genetically-validated species-level entities over both space and time. Additionally, considerable potential for observational error exists in measuring various genitalic traits based upon the degree of animal retraction prior to dissection (Emberton 1989).

Molecular data is also not solely capable of resolving valid species-level entities. For instance, mitochondrial (*e.g.*, "bar-code"; Hebert *et al.* 2003) DNA sequence data is problematic in systems with non-zero rates of hybridization, with resulting phylogenetic trees often informing more about the geographic relationships between samples than their taxonomic relatedness (*e.g.*, Shaw 2002, Rubinoff and Holland 2005). Nuclear DNA can also be fallible, as it is possible

A. Native

B. Naturalized

Figure 3: Height:Width ratio distribution for native and naturalized North American land snails along a natural log-transformed axis.

ecological—to identify the boundaries between biologically valid species-level entities.

The most desperately needed revisionary work appears to be the succineids and the western helminthoglyptids, oreohelicids, urocoptids, and *Ashmunella*. The Succineidae demonstrate profound plasticity in not only shell morphology but also genitalic anatomy, and it seems possible that many currently described species are simple ontogenetic and/or ecophenotypic variants within more cosmopolitan entities. For the western helminthoglyptids, oreohelicids, urocoptids and *Ashmunella*, many 'species' share essentially identical shells and habitat requirements, with diagnoses being largely based on geographic isolation in combination with minor variation in genitalic anatomy. Problematically, not only do we not know how much genitalic variation may be expected within these species, because of climatic variation current levels of population isolation can not be assumed for more than a few thousand years. During full-glacials, for instance, the desert southwest becomes much wetter (Pigati *et al.* 2011), potentially allowing currently isolated mesophile or hydrophile populations to come into contact.

Shell shape and body size patterns

Both the native and naturalized terrestrial gastropod fauna is strongly bimodal in terms of shell architecture, with many species being taller than wide or wider than tall, but with few being approximately as tall as wide. This is a global phenomenon in land snails (Cain 1977).

While native land snails also demonstrate a strongly bimodal and left-skewed interspecific body size distribution along a $\log_2$ axis, native slugs demonstrate a roughly lognormal distribution. Naturalized exotic snails possess a roughly uniform distribution with naturalized slugs being again roughly lognormal. These distributions are atypical within the Animalia, with right-skewed interspecific body size distributions prevailing at regional and larger scales (Kozlowski and Gawelczyk 2002) across a diverse suite of taxa groups including nematodes (Kirchner *et al.* 1980), insects (Siemann *et al.* 1996), fish (Knouft 2004), mammals

through hybridization and recombination for non-coding regions of one species to become sorted with the coding regions (and mitochondrial DNA) of another. An example of this can be found in Nekola *et al.* (2009) in which one Wisconsin *Vertigo nylanderi* Sterki, 1909 specimen with typical shell features and mitochondrial *cytochrome oxidase subunit 1* and *16S ribosomal RNA* (16S) sequences was found to have an *internal transcribed spacer-2* of the *nuclear ribosomal RNA* (ITS-2) sequence characteristic of *Vertigo gouldii* (A. Binney, 1843).

Current and future revisionary taxonomy must, thus, make use of all data—conchological, anatomical, genetic, and

(Brown and Nicoletto 1991), and birds (Maurer and Brown 1988).

Why should terrestrial gastropods behave so differently? First, it is important to note because both native and naturalized slugs have a minimum biovolume much greater than for snails, the lower mode is expunged from the slug body size distribution (Fig. 2). Thus, there is no reason to assume that different sets of rules govern the two groups. Second, the body size distribution of individuals within the land snail fauna demonstrates the expected right-skewed unimodal distribution across all spatial scales (McClain and Nekola 2008). The underlying cause of these unique patterns, therefore, cannot simply represent rarefaction. Rather, per capita evolutionary rate must be at least partially inversely correlated to body size.

Although a number of factors may be at play (McClain and Nekola 2008), the role of dispersal strategy is likely paramount. Land snails generally possess very poor active dispersal abilities, with many individuals moving perhaps no more than 1–10 m over their lifetime (Schilthuizen and Lombaerts 1994, Hausdorf and Hennig 2003) and being unable to actively cross barriers of only 100–1,000 m (Baur 1988, Schilthuizen and Lombaerts 1994). However, small land snails in particular may also possess excellent passive dispersal abilities: members of the genus *Balea* Gray, 1824, for instance, have been repeatedly carried across 9,000 km of open eastern Atlantic Ocean by migrating birds (Gittenberger *et al.* 2006). Successful movement via passive vectors appears to be inversely correlated with land snail body mass for two principle reasons: First, smaller individuals are less likely to be pulled off of vectors by gravity and aerodynamic drag during migration. Second, the ability to uniparentally reproduce—and , therefore, to found a new population via movement of only a single individual—appears to be common in tiny snails such as *Vertigo* (Pokryszko 1987) and *Carychium* Müller, 1774 (Bulman 1990). To establish a new colony, obligatory outcrossing larger snails will require contemporaneous movement of at least two (likely immature) individuals or of a single adult individual that is storing sperm from prior copulatory activities. As a result, large snails are much more likely to experience isolation, restricted gene flow, and allopatric speciation as compared to small snails. It should, thus, not be surprising that while genera of small snails (such as *Euconulus* Reinhardt, 1883, *Gastrocopta, Pupilla, Punctum, Radiodiscus* Pilsbry and Ferriss, 1906, *Vertigo*) share similar faunas across multiple isolated high elevation mesic forests in the desert southwest, species of large snails (such as *Ashmunella, Holospira* von Marten, 1860, *Humboltiana* von Ihering, 1892, *Sonorella, Oreohelix*) living in these same sites tend to be single-mountain endemics (McClain and Nekola 2008).

Underlying Mathematical Mechanisms

Lastly, POwer-law/LOg-normal (POLO; Halloy 1998) distributions were noted for not only the number of species within families and genera, but also for the number of species described by a given researcher. Such distributions are characterized by a hollow "L"-shaped curve in which most entities are rare, but with a very few entities making up the bulk of the sample. Thus, in the native fauna, over ¾ of species are restricted to only ten families, while six families are represented by only single species. And, the top ten genera encompass over 44% of the entire fauna, while 45 genera are represented by only a single species. Likewise, while Henry Pilsbry alone contributed to description of over ¼ of the entire continental fauna, 61 investigators have described only single species. This type of distribution is common to a wide variety of apparently disparate systems, ranging from kinetic energy of gas molecules and earthquake magnitudes, to word use in written works, income distribution among households, citation rates for scientific papers, Internet web hits, copies of books sold, telephone calls received on a single day, lunar crater diameters, solar flare intensity, deaths in wars, wealth of rich people, surname frequencies, city populations, service times for restaurant glassware, and the first marriage age of Danish and U.S.A. women (Preston 1950, 1981, Newman 2005). These all represent complex dynamical systems, which are composed of many components of many different kinds that interact with each other and their extrinsic environment in many different ways and on multiple spatial and temporal scales, giving rise to complex structures and complicated non-linear dynamics (Nekola and Brown 2007). The presence of such POLO distributions both within the North American land snail fauna, as well as within the community of scientists describing this fauna, indicates that both represent complex systems. Those searching for the ultimate mechanisms underlying these patterns should, therefore, consider wisdom gained by not only by biologists and ecologists, but also complexity scientists.

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DNA barcoding cleans house through the Carychiidae (Eupulmonata, Ellobioidea)[*]

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Abstract: The terrestrial, ellobioid taxon, Carychiidae provides an excellent case study for testing integrative taxonomy and addressing the plethora of historical species designations based on vague morphological characters. Since the Carychiidae are ephemeral, hermaphroditic microgastropods (< 2mm) inhabiting permanently moist aphotic zones of epigean (*Carychium* O. F. Müller, 1773) and subterranean habitats (eutroglobiont *Zospeum* Bourguignat, 1856), studies investigating their reproductive system for species delimitation have been futile. Many carychiid species designations were established during the mid-18th to 20th Century heydays of species discovery. Naturalists either split ("splitters") species by recognizing them according to trivial differences in shell morphology or grouped them ("lumpers") based upon common morphological traits. The concept of phenotypic variability was not considered or greatly underestimated in these species hypotheses. Although integration of DNA barcodes has since enabled a reliable identification and delineation of most of the traditional morphospecies, still many morphologically unrecognized evolutionary lineages have been found in apparently widespread and variable taxa. In a retrospective, morphometric approach (*i.e.*, starting from genetically identified, DNA-barcoded material), historically established conchological characters of the outer shell of two European *Carychium* species, *Carychium minimum* O. F. Müller, 1774 and *Carychium tridentatum* (Risso ,1826), are investigated. Revealed was a continuum of intraspecific conchological variability, indicating wide areas of overlap between both taxa. The conventional practice of separating species based on shell morphology alone was sufficient for a qualitative species assignment (*i.e.,* when characteristic phenotypes are observed). Molecular analyses, however, enabled a quantitative species assignment for sympatric populations, intermediate morphotypes and juveniles.

Key words: phenotypic variability, integrative taxonomy, cryptic evolutionary lineages, canonical discriminant analysis, synonomies

ECOLOGY, DISTRIBUTION AND DIVERSITY

Reliable taxonomic assignments have a tremendous impact on the exploration, documentation, and interpretation of biological diversity. Consequently, correct species identification is of paramount importance. Since the phenotype is the first impression we perceive about an organism, its identification is reliant on widely applied and established taxonomic morphospecies concepts. Still, modern-day taxonomy and systematics can take advantage of hindsight. By synergistically integrating independent, but complementary lines of evidence, information for the re-evaluation of century-old species hypotheses can be obtained (Dayrat 2011, Haszprunar 2011). Since the diversification of two evolutionary lineages represents a continuous process, all attributes consulted to infer their delimitation (*e.g.,* behavior, ecology, genotype, and phenotype) should manifest variable degrees of differentiation (de Queiroz 2007). Here,

we present a case history of the conchologically-driven microgastropod taxon Carychiidae (Eupulmonata, Ellobioidea) and initiate a new approach for an updated, integrative taxonomy.

The Carychiidae inhabit moist aphotic zones in leaf litter and rotting wood in fens, mesic forests, and riparian zones (epigean *Carychium* O. F. Müller, 1773) or moist walls and crevices in karst caves (eutroglobiont *Zospeum* Bourguignat, 1856) (Fig. 1). While *Carychium* demonstrates a Holarctic distribution, *Zospeum* was known until recently only from European caves situated in the central Pyrenees, the Cantabrian Mountains, the southern, central Alps and the Dinaric Alps (Gittenberger 1980, Pezzoli 1992, Slapnik 1994, Slapnik and Ozimec 2004). However, Prozorova *et al.* (2011) have documented *Zospeum*-like shell material from the Nodong Cave in South Korea.

Based on conchological criteria, 59 carychiid morphospecies are currently thought to be valid (Bank 2012, R. Bank

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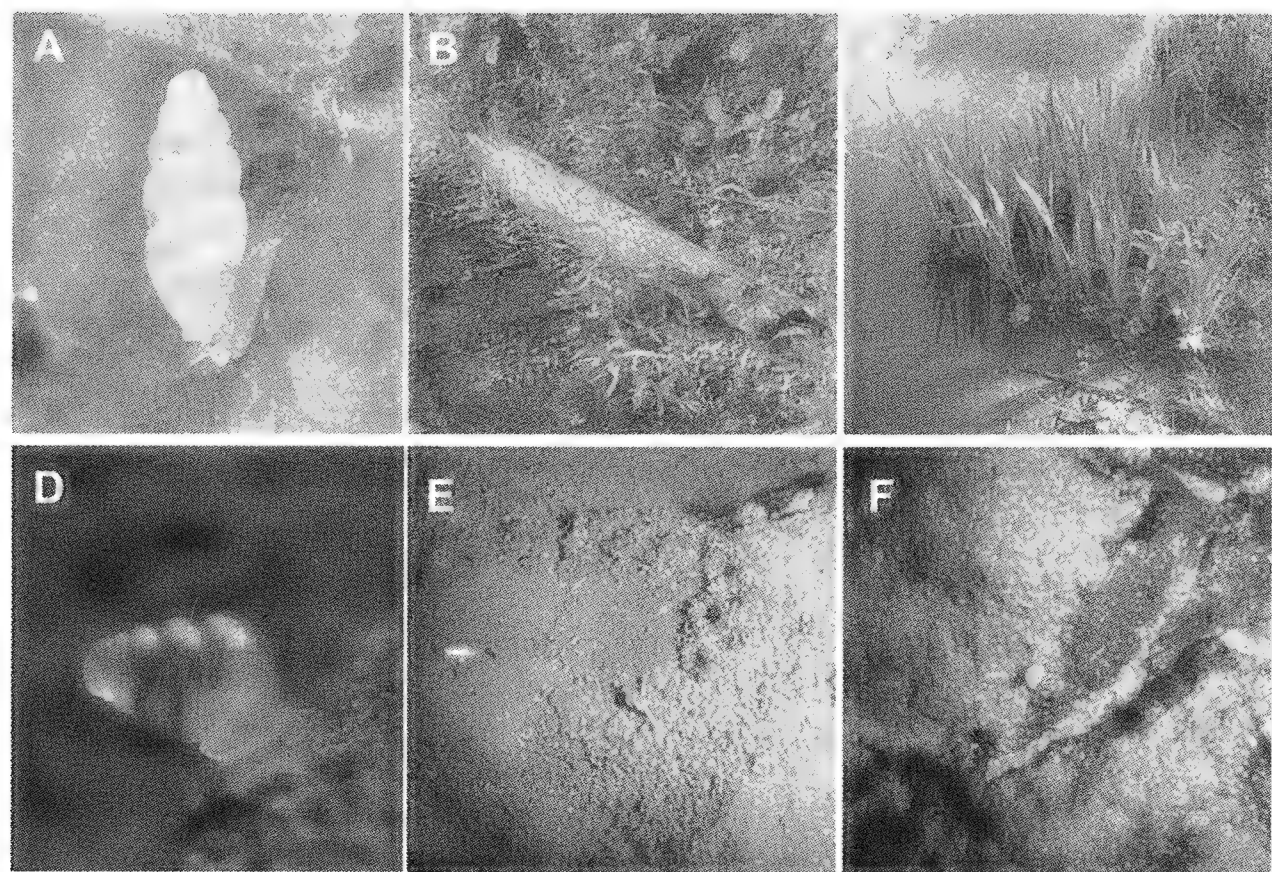
Figure 1. Members and habitat of Carychiidae. **A,** *Carychium* specimen. **B–C,** Typical epigean habitats of *Carychium*. **D,** *Zospeum* specimen (image taken by J. Bedek, HBSD). **E–F,** Typical subterranean habitats of *Zospeum*. Arrows point to specimens. (Color shown in electronic version only).

Figure 2. Description profiles of Carychiidae. Indicated are the cumulative numbers of currently recognized, 'valid' morphospecies hypotheses (y-axis) over time (x-axis). Years of the first and last description are indicated. The dashed lines refer to the 50% and 90% levels of described morphospecies.

and R. Slapnik pers. comm.) (Fig. 2). At the same time, the degree of synonomy (including species and subspecies designations) is more than two-thirds. Notably, the majority of synonomies is linked to the carychiid type species: *Carychium minimum* O. F. Müller, 1774 and *Zospeum spelaeum* (Rossmässler, 1839). Description 'hot-spots' refer to Europe, North America, and East-Asia (Fig. 3). However, these regions reflect taxonomic expeditions, subjective travel routes and interest in malacology rather than true biodiversity. Hence, the finding of two, potentially new *Carychium* species from China (Weigand *et al.* 2013) reflects the collector's botanic itinerary rather than mirror t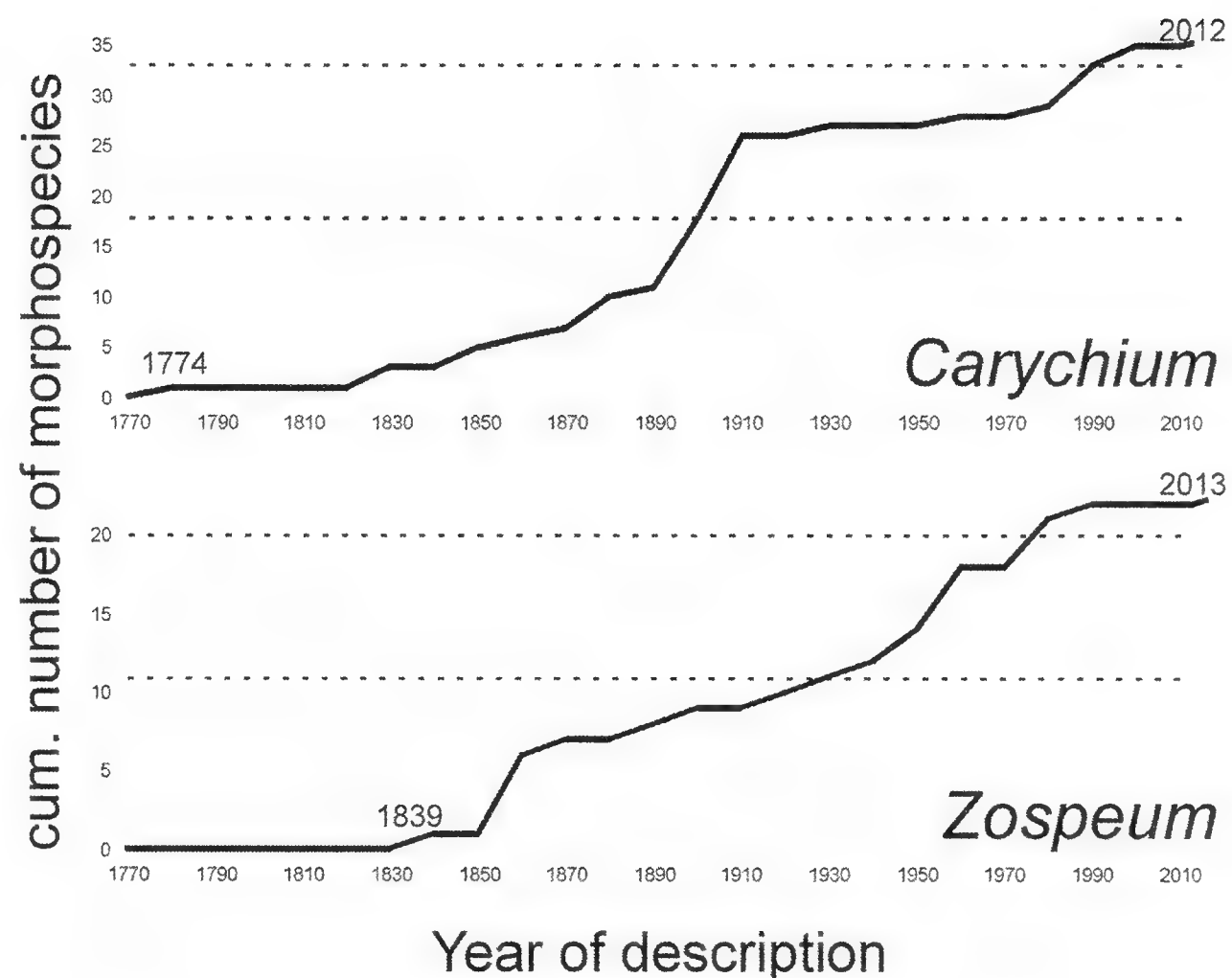he biodiversity of this region. Interest in subterranean biodiversity was ignited when the first cave-dwelling organism was found during the late 18th Century (the blind cave salamander, *Proteus anguinus* (Laurenti, 1768) from Slovenia) (Aljančič 2012), initiating a bonanza of discovery and frenzy amongst taxonomists to describe other cave-dwelling taxa—including *Zospeum*.

CONTINUOUS PHENOTYPIC VARIABILITY

Varying environmental conditions may cause a shift in abundance and distribution of phenotypes for a given species or evolutionary lineage. Phenotypic variability, with overlap between morphospecies, has been demonstrated in numerous gastropod groups such as the Ampullariidae, Cochlicopidae, and Lymnaeidae (Giusti and Manganelli 1992, Estebenet and

Martín 2003, Pfenninger *et al.* 2006, Correa *et al.* 2011, Nekola *et al.* in press). While variable environmental conditions can lead to highly polymorphic lineages, stable environments maintain certain morphotypes and, thus, favor morphological stasis (Wake *et al.* 1983, Weigand *et al.* 2013). This represents a problem for taxonomic and systematic concepts based on gastropod shell characteristics; necessitating a solution to unravel inherent shell plasticity in congruence with the task of distinguishing between intraspecific variability and interspecific boundaries. Usually, a phenotype hypothesis is postulated after taxonomically assessing, sorting, comparing and evaluating a limited number of specimens. However, these formalized phenotype hypotheses only conceptualize the true diversity expressed by all possible phenotypes of a taxon, *i.e.,* the phenome of an evolutionary lineage (Deans *et al.* 2012).

In carychiid taxonomy, standard keys refer to characters such as the level of striation, shell dimensions and proportions, the form and position of the columellar fold as well as the number of whorls (Zimmermann 1925, Pilsbry 1948, Bole 1974, Strauch 1977, Burch and Van Devender 1980, Bank and Gittenberger 1985). It is not surprising that several of these conchological characteristics demonstrate a certain amount of intraspecific phenotypic variability. In particular, the analysis of large datasets of (geographically widespread) specimens provides valuable information. The comprehensive morphometric study conducted by Nekola and Barthel (2002) on North American material of *Carychium exile* H. C.
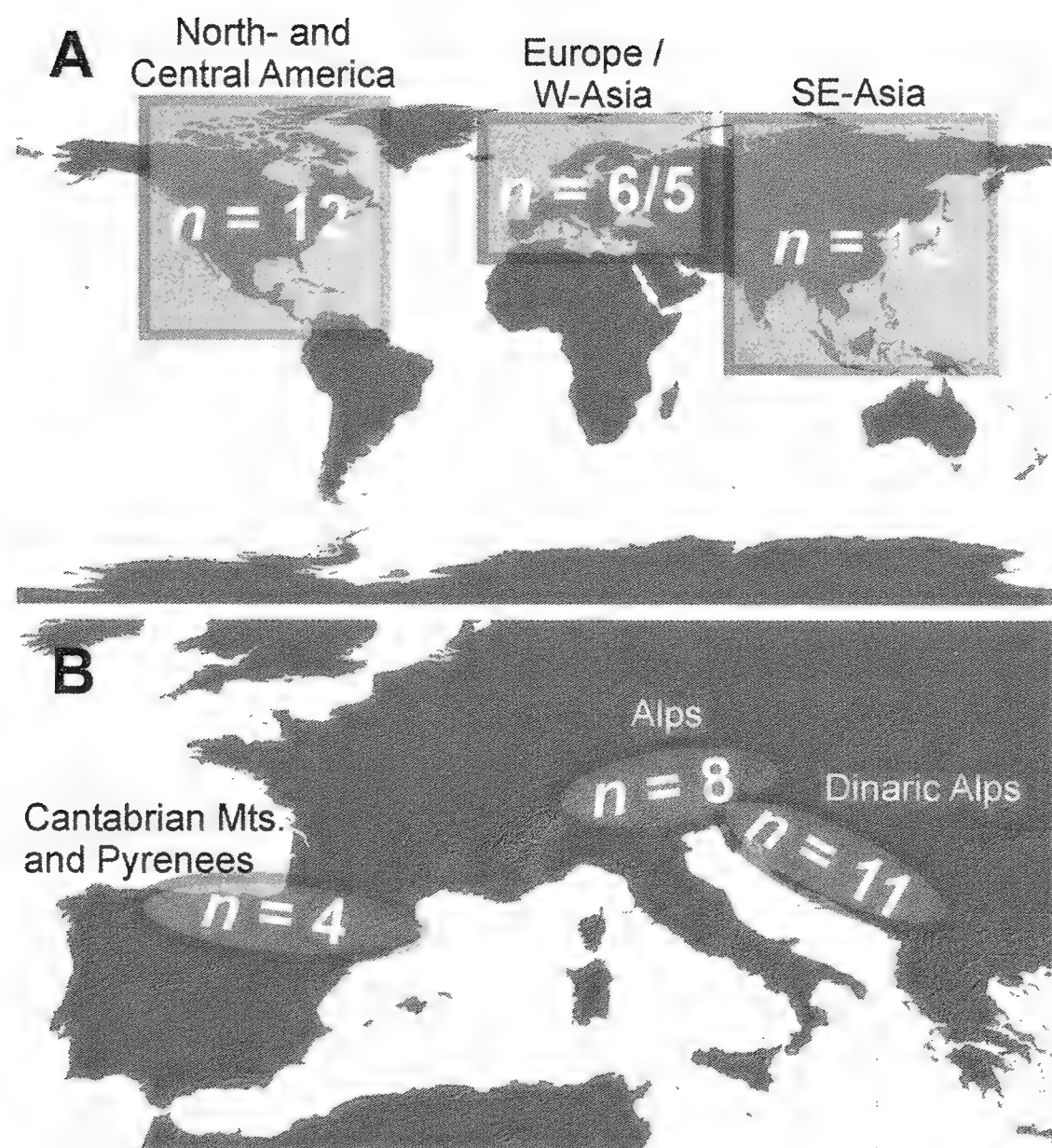
Figure 3. Geographic distribution of Carychiidae. The number of morphospecies (*n*) per geographic region for *Carychium* (**A**) or mountain range for *Zospeum* (**B**) is indicated.

Lea, 1842 and *Carychium exiguum* (Say, 1822) observed a continuous latitudinal variation in shell heights and widths for both species. Geographical variation of shell dimensions and proportions was also illustrated in Zimmermann (1925) on a pan-European set of *Carychium*. Bulman's (1990) analysis of Polish *Carychium tridentatum* (Risso, 1826) revealed a wide variability within and between populations, linking specimens by a continuous phenotype sequence. Most populations demonstrated significant variation in at least two characters, *e.g.*, shell dimensions, shell proportions or whorl number. Moreover, Bulman (1990) emphasized that the interpretation of form and position of the columellar fold depended on the number of whorls and the angle of view respective of the whorl number. In addition to the variable shell shape, comparative anatomical investigations of Carychiidae are complicated by their minute size and seasonal hermaphroditism (Morton 1954, Jochum 2011).

IMPLEMENTATION OF DNA BARCODES

Since the 1960s, molecular data has been implemented to assist taxonomic and systematic surveys (Suárez-Díaz and Anaya-Muñoz 2008). Weigand *et al.* (2011) introduced DNA barcodes, *i.e.*, fragments of the cytochrome c oxidase subunit I (COI) for species identification and delineation in Carychiidae.

The initial study indicated that 90% of carychiid morphospecies designations were supported by molecular data. Additionally, their investigation revealed cases of past taxonomic lumping and splitting due to high intraspecific phenotypic variability and morphological stasis. However, only a few individuals per population and morphospecies were investigated. A more comprehensive survey discussing the evolution of the Carychiidae unraveled a large number (50%) of morphologically unrecognized evolutionary lineages (Weigand *et al.* 2013). A certain discrepancy between conchologically-driven and molecular taxonomy exists (Weigand *et al.* 2011, 2013, Weigand, Goetze *et al.* 2012).

RE-EVALUATION OF CONCHOLOGICAL CHARACTERS OF THE OUTER SHELL – THE SETUP

By using DNA-barcoded specimens in a retrospective approach, we explored the intraspecific variability of shell characteristics within two European *Carychium* species: *Carychium minimum* (*CM*) and *C. tridentatum* (*CT*). Specimens were retrieved from a larger phylogeographical study (Weigand, Pfenninger *et al.* 2012). An unambiguous genetic delineation was possible for all 742 specimens (*CM*: 325, *CT*: 417) in more than 270,000 pairwise comparisons (Fig. 4). Within and between species, genetic variability is separated by a barcoding gap of 3.4–4.3% Kimura-2-parameter (K2P)-distance. After molecular identification, the objective was to clarify the utility of a tractable strategy to augment common taxonomic practice within Carychiidae: the weighing of shell dimensions (shell height 'H', shell width 'W', length of the last whorl 'LW' and length of the upper whorls 'UW') and shell proportions (shell length to shell width 'L/W' and the last whorl to the upper whorls 'LW/UW'). Specimens were measured (*CM* = 161; *CT* = 110) by counting pixels in images (456 pixels = 1.00 mm) between measurement landmarks (Fig. 5). Additionally, shell proportions were calculated. Uni- and bivariate biostatistical analyses of morphometric data for the investigated characters were conducted in GraphPad Prism version 4.03 (San Diego, California, U.S.A.). Both datasets were tested for a Gaussian distribution using the D'Agostino-Pearson test (D'Agostino and Stephens 1986). Subsequently, and due to the fact that both datasets were not normally distributed, the Mann–Whitney–U–test for continuous, unpaired samples was conducted (Mann and Whitney 1947). Box–and–whisker diagrams were applied for visualization of numerical data (Benjamini 1988). The upper and lower whiskers indicate the sample maximum and minimum, respectively. Boxes are restricted by the 25th (lower boundary) and 75th percentiles (upper boundary), including the median.
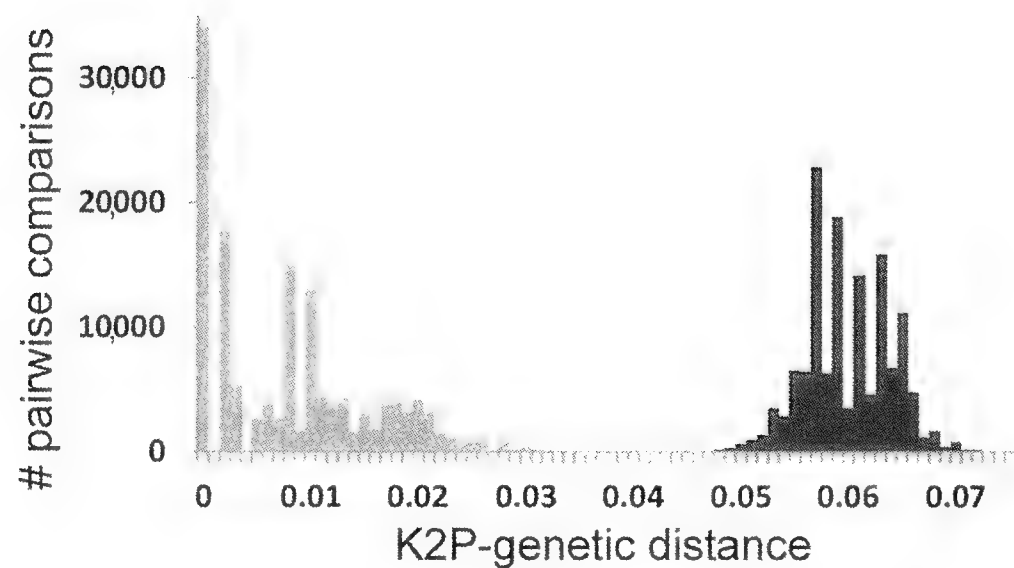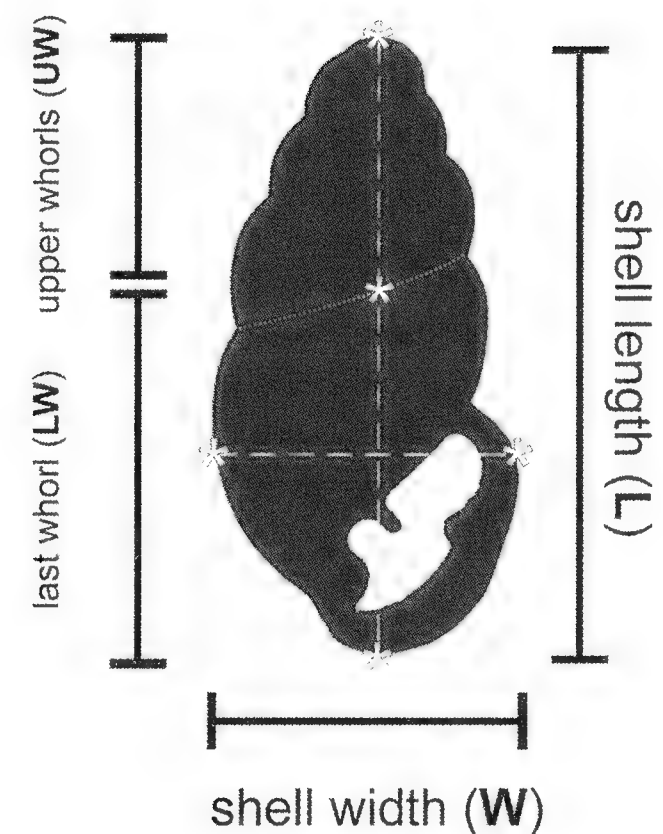
Figure 4. Genetic delimitation. The frequency distribution of the pairwise comparisons for 742 DNA barcoded individuals of *C. minimum* and *C. tridentatum* is shown. Intraspecific (grey) and interspecific (black) genetic variability (as Kimura-2-paramter, K2P-distance) are non-overlapping and separated by a barcoding gap.

Figure 5. Measurement landmarks.

RE-EVALUATION OF CONCHOLOGICAL CHARACTERS OF THE OUTER SHELL – FINDINGS AND CONSEQUENCES

Summarized in Table 1 are the mean values (± SD) of metric shell characters for *CM* and *CT* obtained from this study's genetically-identified material and empirical data extracted from carychiid literature (Zimmermann 1925, Watson and Verdcourt 1953, Bulman 1990). Shell dimensions and proportions demonstrated continuous variability, often indicating a considerable degree of interspecific overlap (Fig. 6). We emphasize that these areas represent defined zones of overlap. Whereas, in the case of potential mtDNA introgression events (Currat *et al.* 2008), one would expect the occurrence of randomly distributed values across the morphospaces of both species. Error-rates, here defined as incongruencies between the molecular identification and a conchological assignment, were inferred by a canonical discriminant analysis conducted in SPSS version 12.0 (IBM, New York). DNA barcode assignments were considered as the fixed variable. At least 8 % of incongruently identified individuals (including all shell characteristics) were revealed for

a classification. An identification procedure relying on shell length only resulted in the maximal error-rate of 34 % (Table 2). In general, the power of discrimination was higher with shell proportions than shell dimensions. This suggests a characteristic allometric shell shape for both taxa for which absolute changes in a given shell dimension are buffered by the proportional change of another dimension (Shingleton 2010). Hence, the allometric shape of characteristic phenotypes is sufficient for qualitative species identification (presence/absence), which is already intuitively performed by the differentiation of a more robust *CM* and a more slender *CT* phenotype (Fig. 7). If a quantitative assessment or acquisition of knowledge about the population structure at localities of sympatry is the primary aim, the complementary application of molecular data is requisite. As demonstrated, DNA barcodes of *CM* and *CT* are non-overlapping and clearly discriminate between intra- and interspecific genetic variability (Fig. 4). We deposited DNA barcodes of Carychiidae in the Barcode Of Life Data system (BOLD; http://www.boldsystems.org) (Ratnasingham and Hebert 2007) in the projects 'Phylogeography of *Carychium*' (PHYCA) and 'Barcoding carychiid microsnails' (BARCA).

Table 1. Mean values for shell dimensions [in mm ± SD] and ratios [± SD] of *C. minimum* and *C. tridentatum* specimens. Mean values of morphometric measurements extracted from relevant literature are given for I: Zimmermann (1925); II: Watson and Verdcourt (1953) and III: Bulman (1990). L: shell length; W: shell width; LW: last whorl; UW: upper whorls.

Shell characters	*Carychium minimum*			*Carychium tridentatum*			
	own data	I	II	own data	I	II	III
L	1.81 ± 0.11	1.80	1.79	1.89 ± 0.10	1.95	1.95	1.74
W	0.92 ± 0.07	0.95	0.87	0.87 ± 0.12	0.93	0.84	0.83
L/W	1.97 ± 0.11	1.90	2.06	2.20 ± 0.17	2.10	2.32	2.09
LW	1.17 ± 0.07			1.09 ± 0.07			
UW	0.65 ± 0.07			0.80 ± 0.08			
LW/UW	1.81 ± 0.20			1.38 ± 0.16			
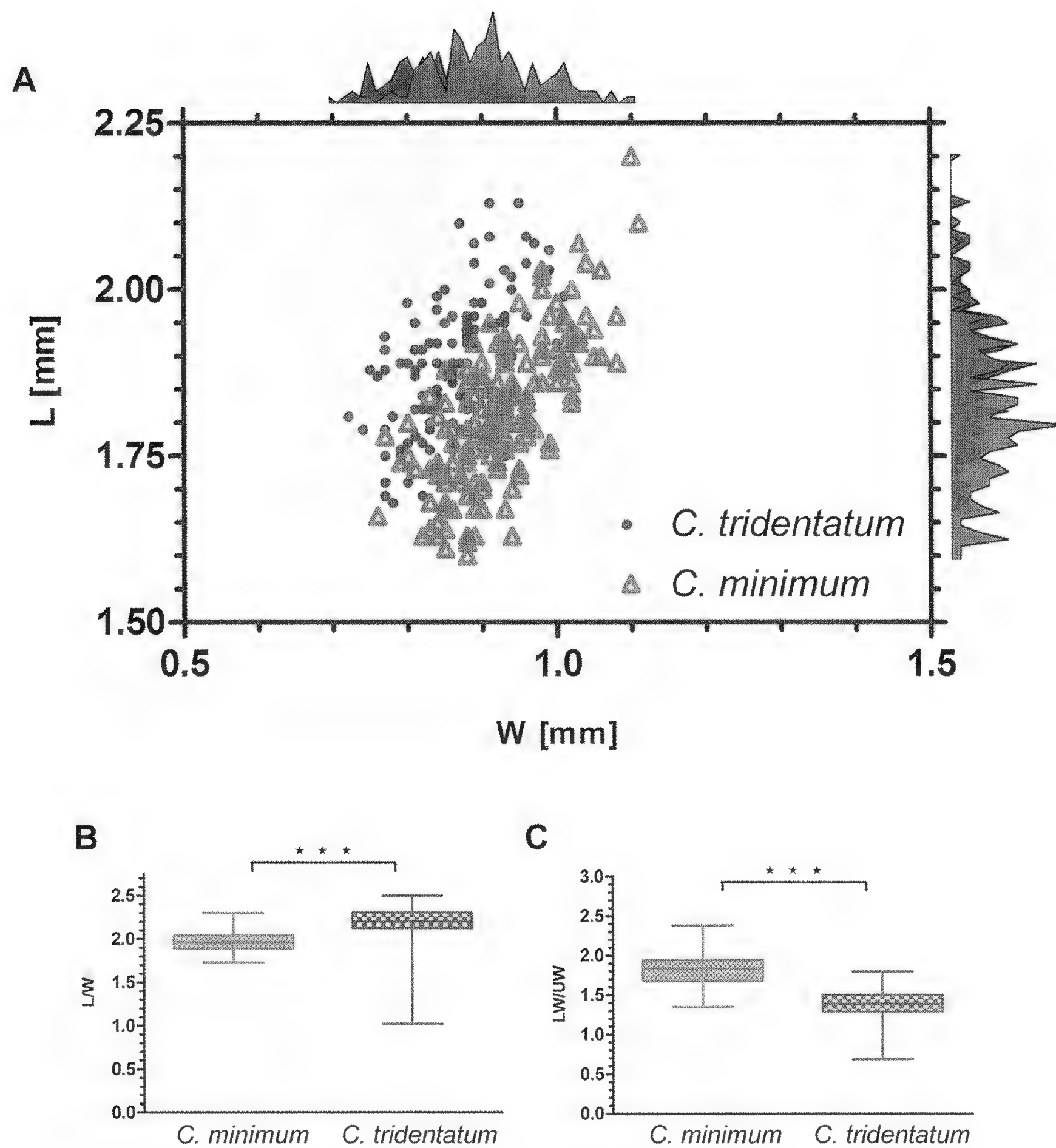
Figure 6. Morphometry. Results of the conchological measurements for *Carychium tridentatum* (blue) and *C. minimum* (red) are given. **A,** Overlapping distributions of shell length (L) and shell width (W). **B,** Box-and-whisker plot for the ratio shell length/width. Both species demonstrate significantly different means. However, all variability of *C. minimum* lies within the conchological range of *C. tridentatum*. **C,** Box-and-whisker plot for the ratio last whorl/upper whorls. Both species demonstrate significantly different means. However, both species possess overlapping distributions. (Color shown in electronic version only).

each (Weigand *et al.* 2011, 2013). In particular, geographically widespread taxa were found as valid proxies in predicting the existence of unrecognized evolutionary lineages in *Zospeum*. Since these species are already listed as vulnerable or endangered (Ozimec *et al.* 2009), knowledge about the existence of (cave-) endemic evolutionary lineages instead of a widespread taxon is of highest conservation value. Pitfalls of DNA barcoding for the delineation of carychiid species arise when incomplete lineage sorting or introgression after hybridization are present (Moritz and Cicero 2004). We regard both as valid risks in cases where two or more morphospecies share (or have very closely related) DNA barcodes. Moreover, the existence of clearly, genetically separated evolutionary lineages (*i.e.,* having distinct DNA barcodes and nuclear DNA) is best explained by the differentiation of isolated populations irrespective of accompanying morphological, ecological or behavioral changes (de Queiroz 2007).

Intraspecific variability of shell characteristics (high for *Carychium*, low for *Zospeum*) was sometimes misinterpreted when postulating former morphospecies hypotheses in Carychiidae. A fact that Burch and Van Devender (1980) in their latest revision of North American *Carychium* acknowledged as follows: "[...] some authors [...] have not treated *C. exile* and *C. exile canadense* as being distinct from *C. exiguum*. Similar problems may exist in the *C. mexicanum, C. floridanum* and southern *C. exile* forms". In particular, assumedly widespread and (moderately) variable morphospecies seem to be affected, *e.g., Carychium pessimum* Pilsbry, 1901, *Carychium noduliferum* Reinhardt, 1877, *Carychium mexicanum* Pilsbry, 1891, *Carychium costaricanum* Von Martens, 1898, *Zospeum suarezi* Gittenberger, 1980, *Zospeum spelaeum schmidtii* (Frauenfeld, 1854), and *Zospeum isselianum* Pollonera, 1887 (Weigand *et al.* 2013). In light of addressing the question of magnitude – the known and the unknown – *i.e.,* how many cryptic species we might expect in respect to our own studies, the situation can be best explained by the morphospecies, *Z. isselianum*. It can be estimated, via information gleaned from the literature (Italian, Austrian, Slovenian, Croatian, and

Contrary to the variable *Carychium* shell, the *Zospeum* shell demonstrates a rather limited spectrum of phenotypic variability. Since the more stable environmental conditions encountered in subterranean habitats select and stabilize for an evolved phenotypic adaptive optimum, cave populations are known to be prone to convergent evolution of phenotypes and commonly feature limited variability (Juan *et al.* 2010). Exemplary is the case of Dinaric *Zospeum*, including seven populations collected at type localities (Fig. 8). DNA barcoding revealed the two morphospecies, *Zospeum isselianum* Pollonera, 1887 and *Zospeum spelaeum schmidtii* (Frauenfeld, 1854) to comprise more than one distinct, and so far, morphologically unrecognized evolutionary lineage

Table 2. Results of the Canonical Discriminant Analysis. A prior grouping of DNA barcoded specimens (*CM*: *Carychium minimum*, *CT*: *C. tridentatum*) was tested given different conchological classifications. Provided are the numbers of congruently ('*CM* as *CM*' and '*CT* as *CT*') as well as incongruently classified specimens ('*CM* as *CT*' and '*CT* as *CM*'). Percent values are shown in brackets. For each variable, the total congruence (T), *i.e.*, original grouping correctly classified, is provided in percent. Canonical correlation = 0.807; Eigenvalue = 1.872; Wilk's Lambda = 0.348; Chi^2 = 287.984; p = 0; df = 4. Prior assignment probabilities were calculated from individual group sizes.

| Variable(s)	CM as CM	CT as CT	CM as CT	CT as CM	T (%)
all variables ('L'+'W'+'L/W'+'LW/UW')	158 (94.6)	98 (89.1)	9 (5.4)	12 (10.9)	92.4
shell proportions ('L/W'+'LW/UW')	158 (94.6)	97 (88.2)	9 (5.4)	13 (11.8)	92.1
last whorl/upper whorls ('LW/UW')	149 (89.2)	100 (90.9)	18 (10.8)	10 (9.1)	89.9
shell length/shell width ('L/W')	153 (91.6)	86 (78.2)	14 (8.4)	24 (21.8)	86.3
shell dimensions ('L'+'W')	149 (89.2)	88 (80.0)	18 (10.8)	22 (20.0)	85.6
shell width ('W')	133 (79.6)	63 (57.3)	34 (20.4)	47 (42.7)	70.8
shell length ('L')	138 (82.6)	46 (41.8)	29 (17.4)	64 (58.2)	66.4

Bosnia Herzegovinian finds) and museum collections housed by the Slovenian Academy of Sciences and Arts in Ljubljana, the Slovenian Museum of Natural History, and the Croatian Biospeleological Society Collection in Zagreb (R. Slapnik, unpublished data), that we are dealing with over 300 localities for this morphospecies alone. If taken one step further, and the 8,000 plus caves in Croatia (Bedek *et al.* 2006) are considered for potential finds of *Zospeum* populations, we soon find we are juggling immense numbers of isolated populations and potentially, morphologically unrecognized evolutionary lineages. We emphasize the sheer magnitude of *Zospeum* biodiversity in all caves of their potential distribution. Since they harbor valuable intraspecific genetic resources, resources that might vanish even if other populations of the species survive (Bálint *et al.* 2011), conservation of these subterranean systems is paramount.

FUTURE DIRECTIVE TOWARDS INTEGRATIVE EVIDENCE

Taxonomic names are often the only link between different data sources. The informative value for all taxonomic characteristics is high when character states are well separated and easy to identify. If taxonomy is ambiguous or wrong, downstream analyses may be considerably affected. Taxonomic errors greatly affect interpretation of a) the richness, distribution and abundance of taxa, b) the taxonomic age estimates retrieved from the fossil record, c) the reconstruction of paleovegetation types based on ecological requirements of gastropod species, d) bioclimatic niche models constructed from digital occurrence data, and e) outcomes of monitoring and conservation studies and lastly, management and protection strategies of species.

We strongly encourage employing multiple lines of evidence in postulating species hypotheses. Ambiguous (morphological) taxonomic identification is not confined to a certain group and many taxonomists are faced with similar issues (*e.g.*, Porco *et al.* 2012). For this reason, an integrative taxonomic approach is needed. In addition to other considerations, the following points can help taxonomists to avoid ambiguities associated with investigations of single characteristics: 1) Integration of multiple datasets (*e.g.*, morphology, ecology, genetics, geography, reproductive isolation), 2) collection and (morphological + molecular) characterization of type locality populations to link historic morphospecies hypotheses and genetic data, 3) non-invasive DNA isolation to preserve voucher specimen shells (*e.g.*, by foot tissue clipping (Haskell and Pan 2010) or as performed in Weigand (2013)), 4)
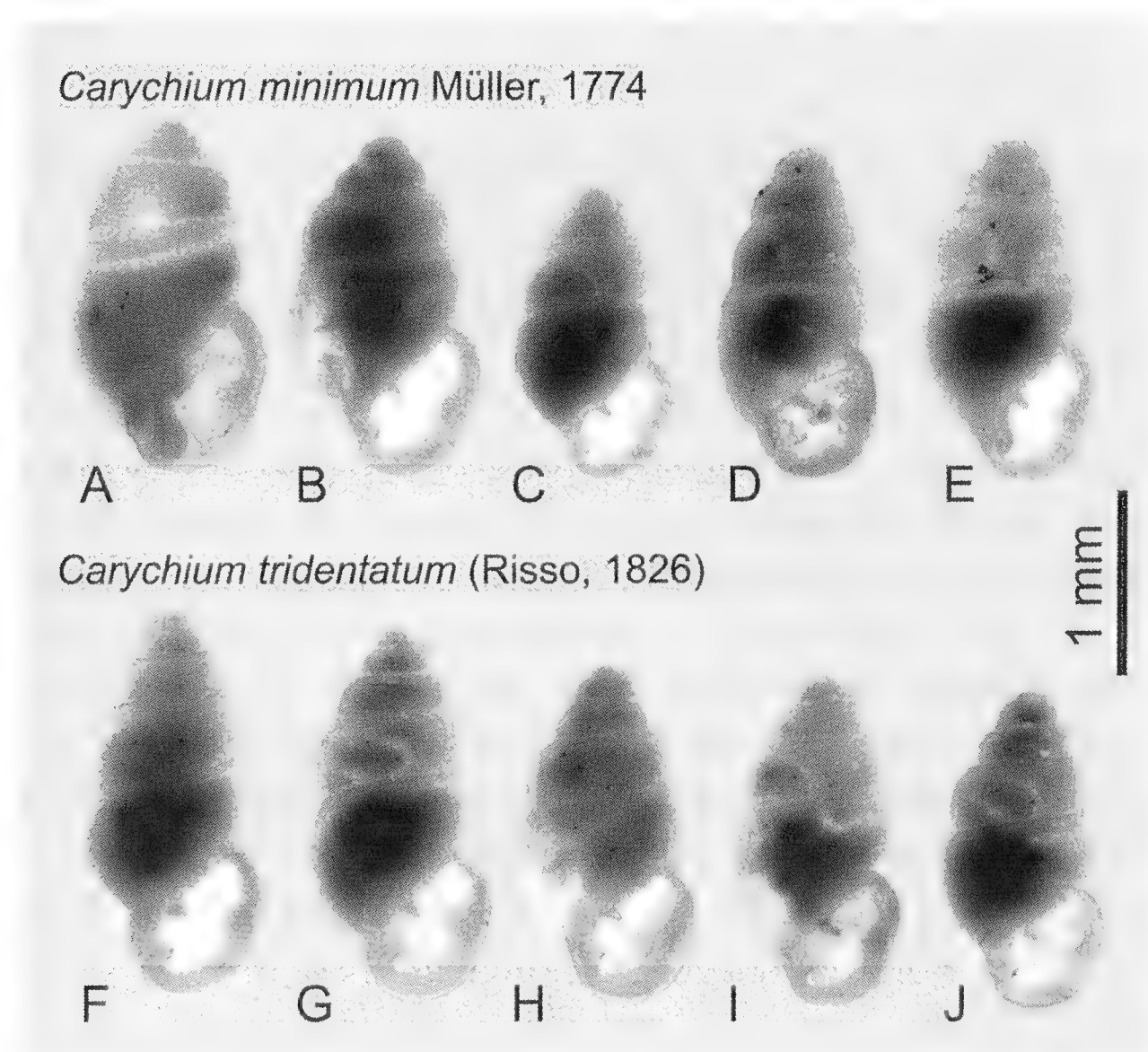
Figure 7. Intraspecific variability of *Carychium* phenotypes. The most characteristic (to the left) and intermediate phenotypes (to the right) are illustrated for the two species *C. minimum* (upper row) and *C. tridentatum* (lower row).
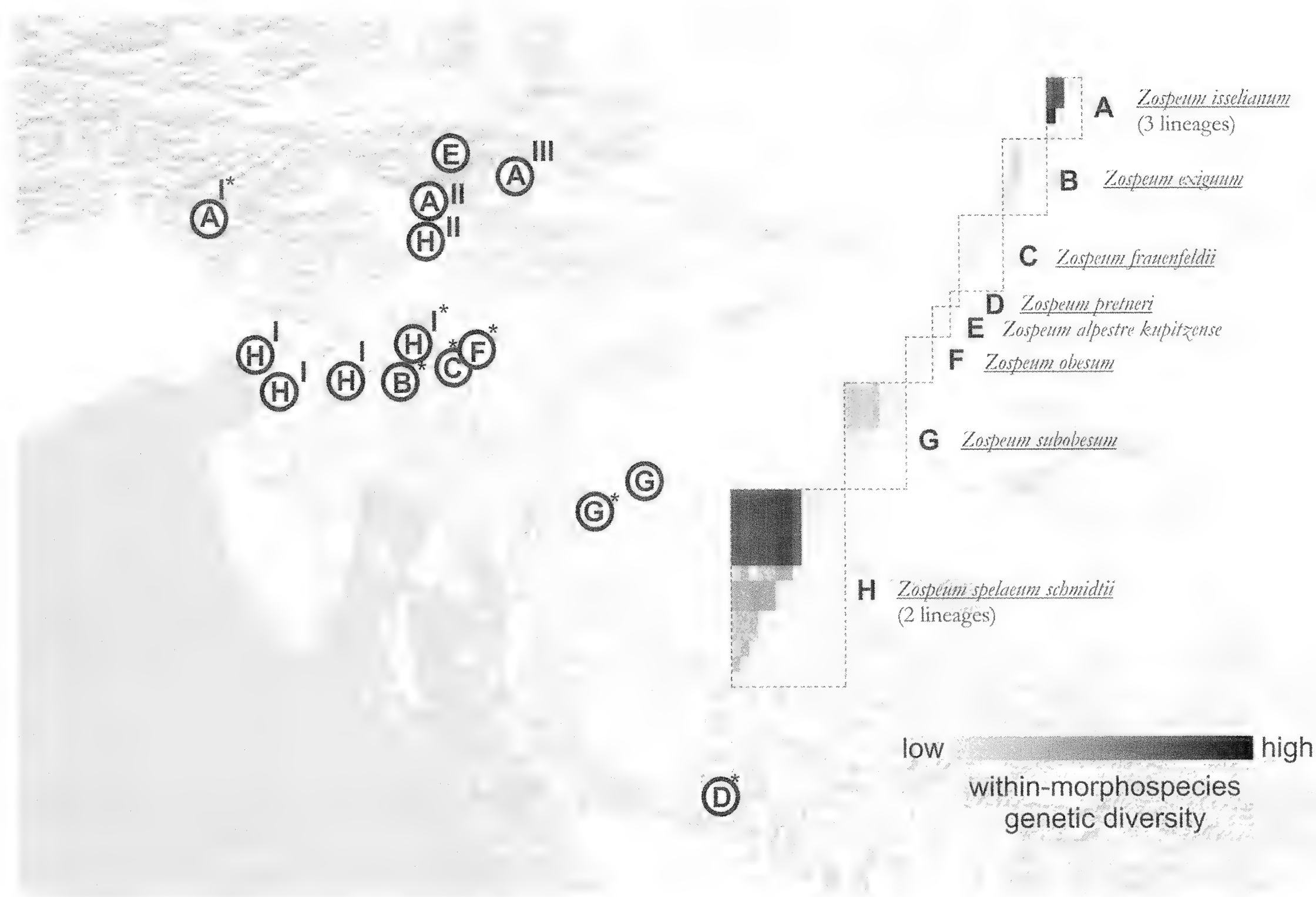
Figure 8. Dinaric *Zospeum*. Geographic distribution and genetic delimitation of Dinaric *Zospeum* morphospecies (modified after Weigand *et al.* 2011, 2013). Evolutionary lineages, including specimens from the type locality, are underlined. Type localities are designated by an asterisk. The heat-map indicates intra-morphospecies genetic diversity (light grey: low; black: high). Distinct cave populations of the morphospecies *Z. spelaeum schmidtii* and *Z. isselianum* are also genetically identified as separate evolutionary lineages (I–III).

analyses in conjunction with the examination of type specimens, and 5) deposition of data sources to ensure repeatability (*e.g.*, voucher specimens in museum collections, DNA barcodes in online databases, biological datasets in Dryad or phylogenetic tree hypotheses in TreeBase). The "cyber-centipede" *Eupolybothrus cavernicolus* Komerički & Stoev, 2013 may serve as an example (Stoev *et al.* 2013).

Currently, the authors are working on an integrated revision of the Carychiidae, investigating specimens from type localities, museum material including types, and implementing morphological and molecular datasets as well as a new imaging technology (nanoCT scanning) (see Jochum *et al.* 2013). Specifically, we are focusing on the columellar fold, since this character has long been established as a diagnostic trait in carychiid taxonomy (Watson and Verdcourt 1953, Lozek 1957, Strauch 1977, Burch and Van Devender 1980, Bank and Gittenberger 1985). However, its information content must be re-evaluated (see *e.g.*, Bulman 1990) by using different datasets.

In conclusion, the Carychiidae were not spared the species lumping and splitting tendencies of earlier taxonomists.

Notably, apparently widespread morphospecies, fossil taxa and descriptions of often highly endemic (sub-) species may be greatly affected, resulting in potential, long-term identification failures (Villa and Villa 1841, Pilsbry 1891, 1894, Clapp 1906, Zimmermann 1925, Strauch 1977). In one case, Pilsbry (1948) stated that the "two specific names introduced by Bourguignat seem to have been based upon selected large (*existelium*, length 2 ½ mm), and small (*euphaeum*, length 1 ¾ mm) specimens [...] Both were described as smooth, and without doubt are synonymous with *C. exiguum*". In addition, the astonishing discovery of nine *Carychium* species (including two first descriptions) at a single fossil site in Poland (Stworzewicz 1999) could be interpreted as an alternate or successive occurrence of distinguishable states of a few evolutionary lineages. Although all these problems have been widely reported, naturalists, conchologists and malacologists are still churning up new species without due consideration of today's multifaceted advances in taxonomy, as in the case of *Carychium arboreum* Dourson, 2012 ("The shell is 1.8–2.1 mm in height, pupa-shaped [...]") (Dourson 2012). The integration of molecular data along with its potentiality

to flag cases of cryptic species further enables taxonomists to better evaluate the degree of information derived from traditionally-applied conchological characters. Moreover, after cryptic species are flagged, in-depth conchological investigations may well reveal so far overlooked, diagnostic characteristics congruent with the molecular delimitation, rendering the species pseudo-cryptic. On the other hand, it should be emphasized that much valuable knowledge about the distribution, ecology and species diversity of carychiids has been gleaned from amateur scientists. In the final analysis, only a cooperative, integrative approach will enable us to know what we treasure and what we are losing in the long run.

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Poorly explored jewels of the tropics: Estimating diversity in non-pulmonate land snails of the family Helicinidae (Gastropoda: Neritopsina)*

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Abstract: Helicinids represent a family of tropical land snails with a distribution range limited to the subtropical and tropical zones of the New World, Australasia, and the Pacific. For an estimate of diversity in this poorly systematically revised group, the total number of described taxa was determined and used for calculations based on analyses of selected case studies with regard to the percentage of valid and new taxa.

Extensive bibliographic searches identified about 1,250 available names, regardless of rank, that were described from 1801 onward. Fifty-eight percent of the names represent New World taxa whose majority (63%) was created before 1880 while the intensive study in most of the Australasian and Pacific areas started much later with the bulk of taxa described between 1880–1930. An analysis of the distribution of the type localities and the times of descriptions allowed for identification of scarcely- and well-studied areas.

Eight potentially representative case studies of major revisions were compared with respect to changes in described versus "true" diversity. The geographic range covered Costa Rica, Cuba, Jamaica, Lesser Antilles, New Caledonia, northeast Australia, and the Hawaiian and Gambier Islands. In these studies approximately half of the available names were regarded as synonyms (range from 37 to 72%). On the other hand, 36 to 41% of the recognized diversity represented new species depending on whether a more lumping or splitting approach was considered, the latter simulated by simply counting subspecies as equal units of diversity. The amount of new taxa ranged from 2% (Cuba) to 90% (Gambier Islands). Under the assumption that six of the studies were representative throughout the area of distribution, worldwide diversity would range from 770 to 1,140 species or up to 1,400 species if the studies from the Australasian-Pacific area were realistic. Although obviously poorly studied, in comparison with an estimate for all continental molluscs of Mexico and Central America by Thompson (2011), helicinids would still be among the better documented snail families for this region.

The following aspects and their consequences are discussed as most significant drawbacks in the exploration of helicinids: questionable systematic concepts above species level; limited recognized differentiating characters and convergence; species complexes and last, massive habitat loss, increasingly fragmented distribution and extinction. Another practical aspect is the rather limited availability of wet preserved material.

Key words: systematics, alpha-taxonomy, research history, synonymy, distribution

This contribution aims to provide a realistic estimate on the diversity of the family Helicinidae. Helicinidae together with the closely related Proserpinidae and Proserpinellidae represent an independent evolutionary branch to a terrestrial mode of life that rarely conquered drier habitats. The main distribution of helicinids stretches throughout the tropics of the New World, Australasia, and the Pacific. They are absent in Africa and on the Indian subcontinent with a single species occurring on the Seychelles (Fig. 1). Among the families mentioned only helicinids underwent some spectacular radiations, *e.g.*, the Stoastomatinae on Jamaica, and reached a recent diversity comparable to some large pulmonate families. Clausiliidae, for example, a family that evolved the unique clausilium as closing device, comprise nearly 1,300 recent species (Nordsieck 2007) which might be about 1.5 times helicinid diversity.

A well-founded assessment of the diversity of helicinids would require a revision of all taxa described with current state-of-the-art methodology and sufficient sampling efforts in all regions of the distribution. In a group of organisms of no given economic or nutritional value, with its main distribution in tropical countries with a very limited local systematic workforce and which never seriously attracted the attention of


Figure 1. Distribution of the family Helicinidae (in black), partially generalized to political units disregarding habitat suitability, small islands enlarged as dots.

collectors or amateur scientists, this undertaking is unrealistic. Therefore, the current study explores the amount of divergence of theory to actual knowledge by analyzing the exploration of the family over time, by reviewing selected case studies and identifying well- and poorly-sampled areas. Furthermore, it will examine the specific difficulties in helicinid systematic research and the reasons for taxonomic confusion.

HISTORY OF HELICINID RESEARCH

The first helicinid was described in 1801 by Lamarck (*Helicina neritella* from Jamaica) relatively long after the introduction of the binomial nomenclature by Linnaeus (1758); that is probably due to the tropical distribution of the group. The major phase of species descriptions (Fig. 2) is linked to the scientific output of the German malacologist Louis Pfeiffer who took a strong interest in the family. He described
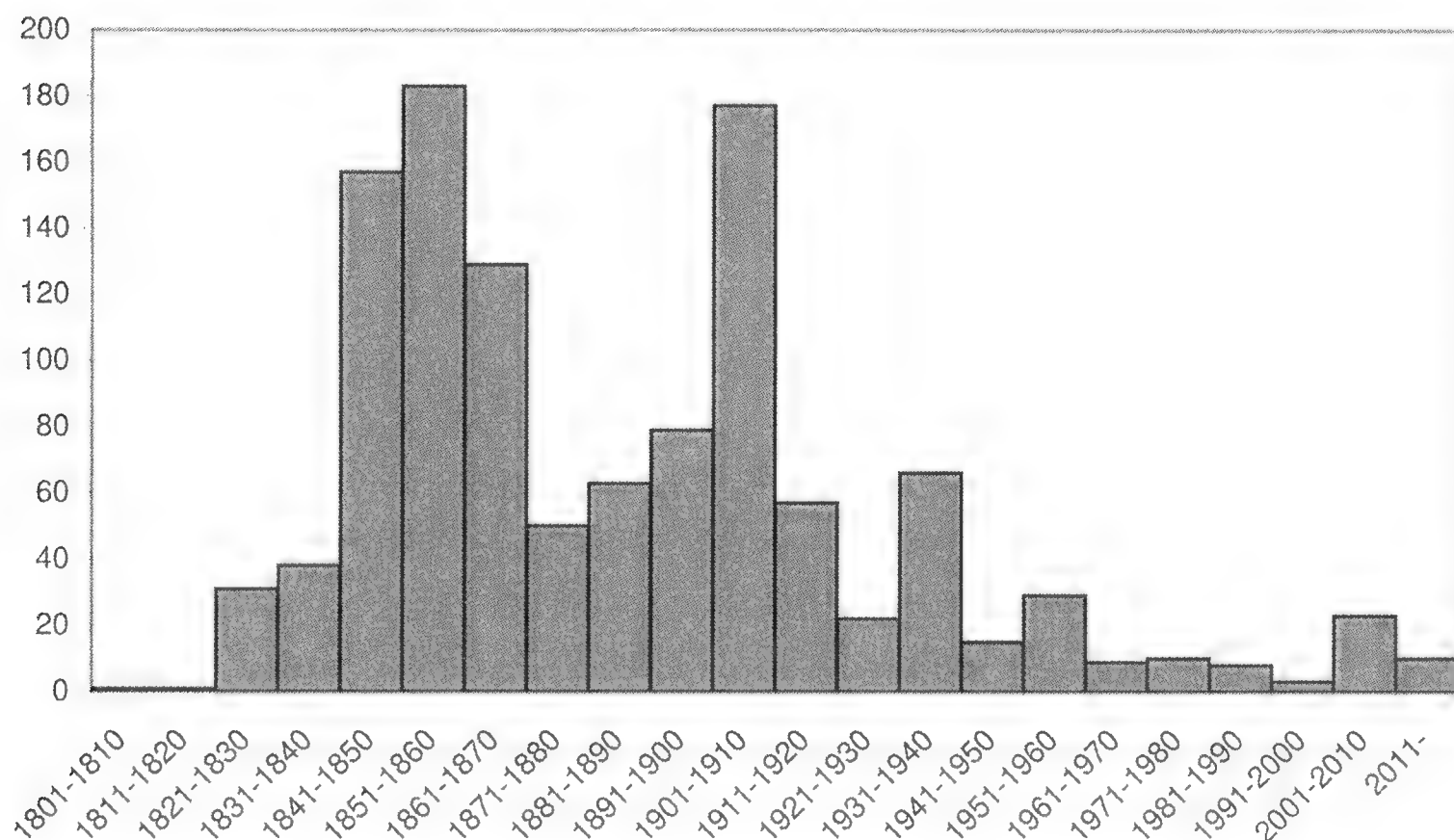
Figure 2. History of research—number of new helicinid taxa described per decade.

more than 170 helicinid taxa which is unsurpassed by any other author. In addition, Pfeiffer (1850–1853) wrote the first helicinid part of the famous *Martini and Chemnitz Systematisches Conchylien-Cabinet* and continued to provide detailed and updated species lists in the four volumes of the *Monographia Pneumonopomorum viventium* (Pfeiffer 1852, 1858, 1865, 1876). The helicinid chapters in the *Thesaurus conchyliorum* (Sowerby 1842, 1866) constitute the only other significant comprehensive publications of this period although short in text. The presentation in Reeve (1873) is mentioned for completeness, but added little information due to the rather poor quality of the drawings.

Species descriptions kept accumulating after Louis Pfeiffer's death in 1877 although at a lower rate and the new edition of the *Martini and Chemnitz Systematisches Conchylienkabinett* (Wagner 1907–1911) was the appropriate occasion for a new and the last comprehensive and illustrated presentation of the entire family. In the absence of an expert for the family, Kobelt motivated Antoni Józef Wagner—otherwise working on European land snails—to explore this exotic family (Richling 2005a). Wagner's monograph (1907–1911) and two pre-studies (Wagner 1905a, b) included another 135 new taxa entirely based on material studied in museum collections or gathered by exchange from other malacologists of his time. He recognized 529 species and subspecies, but overlooked another 137 taxa (Fulton 1915). Subsequently the description of new taxa basically slowed down in the 20th century: Pilsbry with new species from nearly the whole Neotropical range of distribution, Aguayo—often co-authored by Jaume—on Cuban material, and Neal (1934) in the revision of the Hawaiian helicinids were the most prolific authors of this period.

METHODS

For the search of helicinid taxa, in addition to the helicinid literature mentioned in the introduction, sources such as the *Zoological Record*, Sherborn (1922–1931), and Ruhoff (1980) were used. This compilation was complemented by the analysis of more than 500 primary and secondary publications.

In counts of taxa obvious misspellings or errors, homonymous *minor*, *major*, etc., and unavailable names were excluded. For subspecies, the nominal subspecies were included in the number of species and only additional subspecies were counted separately. For practical purposes of the present analysis, the geographic attribution of names was simplified to a single political unit in case of multiple type localities. Equally, it does not strictly follow the original description but was adjusted to the present knowledge, *e.g.*,

when a species was described with unknown or an erroneous origin, but can be localized now. The systematic arrangement follows Bouchet and Rocroi (2005).

The case studies explored were selected by the fulfillment of the following criteria: 1) approach on the level of a revision, 2) realization of extensive field work or study of significant collections, 3) study of type material, and 4) coverage of a meaningful political or geographical entity. In addition, a wide range of different specific circumstances and geographical coverage was included.

RESULTS AND DISCUSSION

Available names, geographic distribution, and time scale of explorations

The search for described taxa resulted in approximately 1,250 available names for recent Helicinidae regardless of their rank and nearly 30 names for Proserpinidae and Proserpinellidae *sensu* Thompson (1980, as Ceresidae). About 720 names (~ 58%) were created for New World helicinids including the Galapagos representatives that seem to be more closely related to the Pacific species (Wagner 1907–1911). Roughly 500 taxa (about 40%) cover the Australasian and Pacific helicinids. Only 23 taxa still lack a clear geographic attribution (unknown or too vague like "Pacific" or "South America").

Although an extensive search of literature was performed it is likely that a few descriptions in local journals and little-used languages remain overlooked, but their number is assumed to be rather small. In a less intensive search for new marine taxa that was counterchecked a year later, Bouchet (2006) reported the insignificant amount of only 1.6% uncaptured names.

The major phases of exploration were highlighted in the research history. Typically, the description of species started slow, but along with growing settlements, trade, and further expeditions in the tropics a boom of discoveries followed (Fig. 3). The curve shows two major points of deceleration, the first around the 1880's, that parallels the death of Louis Pfeiffer, and a second around 1930, before it enters a slow phase and more linear trend in recent years.

Although the rate of descriptions dropped, an approach to the saturation zone as it seemed towards the end of the last century is not yet apparent. The time between the two points of slowdown includes the major contributions by Ancey, Bartsch, Möllendorff, Pilsbry and the work by Wagner (1905a, b, 1907–1911). The "jump" between 1930 and 1940 is caused by a single paper, *i.e.*, the revision of the Hawaiian species by Neal (1934) including a high number of intraspecific taxa. More than 50% of all taxa were described by 1880, about 60% by the end of the 19th century, and more than 85% by 1930. For a further analysis in a geographical context, it seems helpful to differentiate roughly three intervals, 1800–1880, 1880–1930, and 1930–recent, to understand the time scale as well (Figs. 4 and 5).

In the New World the highest number of taxa concentrates on the Greater Antilles with Cuba only slightly ahead of Jamaica (Fig. 4). However, more than half of the Jamaican taxa are constituted by the Stoastomatinae that underwent a major radiation only on this island. Therefore, comparable named helicinid diversity on Jamaica lies in the same range as Hispaniola or the entire area of Mexico. Potential diversity reflected in taxa described shows higher numbers on islands with usually endemic species whereas diversity is lower on the mainland of Central and northern South America where described taxa are "randomly" split up in different political units although species distributions cross these borders. The distribution of the latter taxa is, therefore, superimposed by effects of research intensity and the onset of discovery. Countries explored earlier will have more type localities than later investigated areas which is clearly shown for *e.g.,* Nicaragua, Panama, Honduras with very few taxa and most explicitly Belize with no taxon described from its area. But according to personal observations in the collection of the Florida Museum of Natural History, Gainesville (unpubl. data) at least seven helicinid species occur in Honduras while Thompson (2011) only extracted records of two species from literature reviewed by him.

The West Indies—except for Hispaniola and the Bahamas—with neighboring areas of South America and the suitable parts of North America and the northern Central America received the earliest attention by helicinid authors. In fact, the three earliest described species originate from Jamaica and the Unites States. Southern Central America, vast parts of South America and smaller islands were studied much later and obviously constitute a major source for new species, *e.g.,* Costa Rica (Richling 2001, 2004a) and Colombia (Hausdorf 2006).
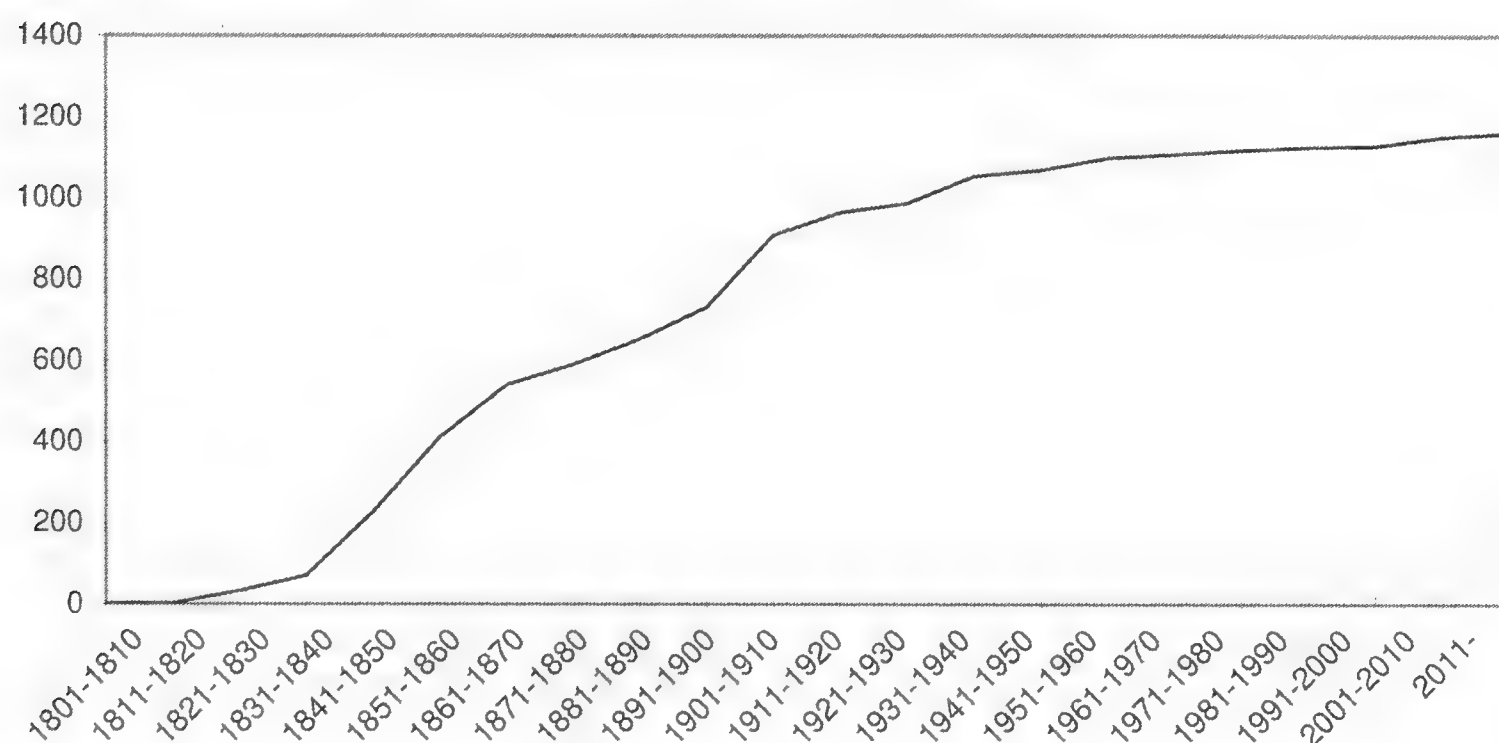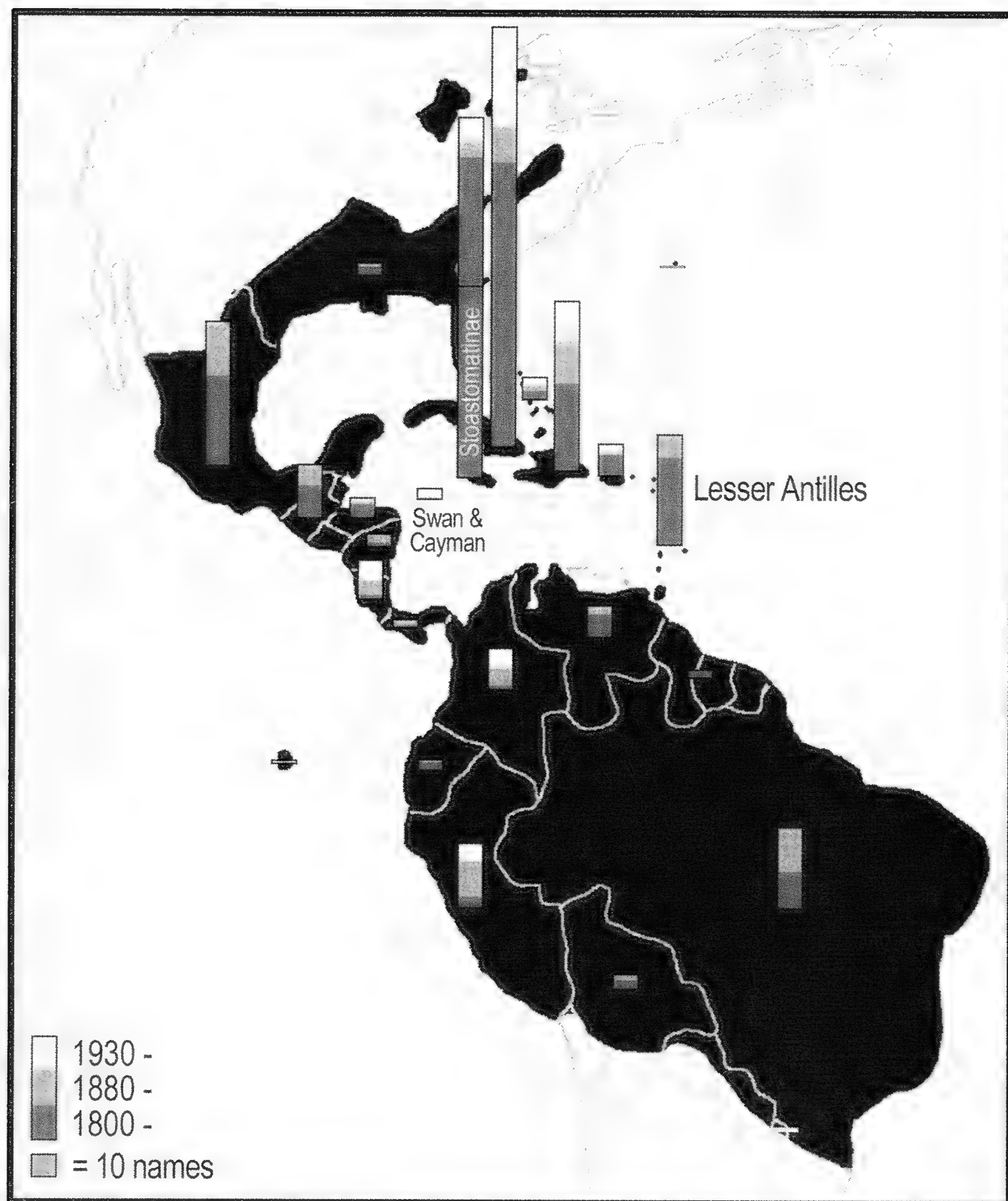

Figure 3. Accumulation curve of newly described helicinid taxa over time.

Figure 4. Numbers of described taxa per indicated political or geographic entity in the New World; maps based on Fig. 1.

absence of any newly described taxa for certain areas points to a lack of serious, more recent studies and marks potential for still unrecognized diversity. This is certainly true for Mexico, Brazil, etc. in the New World and proven for the Lesser Antilles as an area with a long research history (see below). On the other hand, these areas are more likely to be over-described with a certain percentage of names disappearing into synonymy (see below). In more recently explored countries it is likely that the species accumulation curve did not yet reach the saturation zone, because late exploration also implies complex natural settings with still undiscovered diversity, *e.g.*, known for Costa Rica, see below, otherwise likely for parts of South America, Hispaniola and most of the Australasian and Pacific area where suitable habitat is left.

Described diversity versus "true" diversity

In the following section, selected case studies will be reviewed for the relation of described diversity to previously unknown species and the specific circumstances. A numeric summary is given in Table 1.

Costa Rica

Prior to the 1990's the Costa Rican species were poorly sampled and in need of revision. Richling (2004a) based her revision on extensive own field work and the mollusc collection of the Instituto Nacional de Biodiversidad de Costa Rica (INBio) with a good representation of recent material. The study combined morphological characters with distribution patterns and followed a rather conservative approach on the continuum of lumping to splitting. Out of 19 available names, eight species and one subspecies were accepted as valid. Seven new species and two new subspecies were described. Five taxa reported for the country were misidentifications of extralimital species. In general, species ranges turned out to be more restricted than previously assumed.

Remote regions of the remaining wooded areas of Costa Rica still have to be regarded as poorly explored and micromolluscs (smaller than 3-4 mm) are insufficiently covered. Ongoing field work of this author revealed at least two more species of small-sized helicinids that await description.

Cuba

Clench and Jacobson (1968, 1971a, b) and Boss and Jacobson (1973, 1974) revised major groups of the Cuban helicinids (*Viana* H. and A. Adams, 1856; *Emoda* H. and A. Adams, 1856; *Glyptemoda* Clench and Aguayo, 1950; *Calidviana* Baker, 1954; *Ustronia* A. J. Wagner, 1908; *Troschelviana* Baker, 1922;

The Australasian and Pacific area of distribution generally received attention much later. More than 50% of the taxa were described in the second interval of discovery. Only 32% had been named before 1880 while at the same time in the New World 63% of the names were created. The earliest descriptions focused on Polynesian islands and the adjacent Fiji and Vanuatu archipelagos with exception of the Hawaiian Islands. A smaller share of New Caledonian, Australian taxa and of those from the coastal areas and islands of the Indian Ocean were described early as well. The second phase is characterized by the recognition of most of the helicinid diversity of Japan, continental Asia, the Philippines, Indonesia, Papua New Guinea and the Solomons. Most recent publications concentrate on the Ogasawara Islands of Japan (mainly Minato 1980), Australia (Stanisic *et al.* 2010), New Caledonia (Richling 2009), and far eastern Polynesian islands (Preece 1998, Richling and Bouchet 2013).

In the comparatively poorly-studied helicinids it is unlikely that the majority of regions—and more so tropical areas—are entirely explored for new species. In general, the

Figure 5. Numbers of described taxa per indicated political or geographic entity in the Australasian and Pacific area, Hawaiian and most of the Polynesian islands not in true geographical position; maps based on Fig. 1.

Semitrochatella Aguayo and Jaume, 1958; *Alcadia* Gray, 1840, and *Lucidella* Swainson, 1840). They accepted 61 species and 10 subspecies out of 150 names available and described one new species and two subspecies. Unlike the majority of other countries Cuba has a long tradition in field malacology starting with the remarkable explorations by the German Johann Christoph Gundlach, whose land snail collections were mainly investigated and described by L. Pfeiffer (Richling and Glaubrecht 2008). After the major revisions mentioned above only one additional helicinid species was described to date (Sarasúa 1976).

Jamaica

The Jamaican land snail fauna is currently being studied by Rosenberg and collaborators who undertook major collection efforts between 1999 and 2002 with 607 major sites throughout the island (Rosenberg 2005, unpubl.). Jamaican helicinids were first intensely studied by C. B. Adams (mainly 1849, 1850a–c), followed by localized, but "incidental" collections by Baker (1934 a, b). According to the online published working list by Rosenberg and Muratov (2005, unpubl.), 40 species and 13 subspecies of helicinids other than Stoastomatinae are recognized out of 74 names. For the latter subfamily—treated as Stoastomatidae by the authors—75 species and one subspecies are accepted out of 82 available names. Because the Stoastomatinae underwent such an outstanding radiation only on Jamaica and, therefore, represent a special case, they are excluded from further calculations of diversity estimates.

Lesser Antilles

The Lesser Antilles represent a previously rather poorly sampled area that was never studied entirely because of the different political affiliations. Revisionary work on the helicinids is still in progress (Richling 2005b), but the unpublished data suggest that 23 species are valid out of 42 names available and four species await description.

Biogeographic relationships are still poorly understood and the application of new systematic concepts (Richling 2004a) reveals different distribution patterns of more closely related groups than previously assumed. Some taxa currently regarded as single species occur on different islands while others represent island endemics characterized by distinct shell morphology. While for the latter recognition at the species level is fairly certain, the more widely spread taxa could equally comprise "cryptic" species that for unknown reasons did not diverge recognizably in morphology and would add to the helicinid diversity in the Lesser Antilles. These questions require molecular analyses.

New Caledonia

Although Franc (1957) and Solem (1961) provided compilations on the terrestrial snail fauna of New Caledonia, both authors only more or less critically summarized the helicinid species reported for the area. Richling (2009) provided the

Table 1. Previously known and unknown helicinid diversity after revisionary work for different areas compared to the number of available names and records for the area regardless of rank. For data sources and the additions for NE-Australia see respective paragraphs. n. = new, sp. = species, ssp. = subspecies.

Area	Number of available names	In respective revision, number of				Percentage of			
		accepted sp.	accepted ssp.	n. sp.	n. ssp.	valid sp.	valid sp. + ssp.	n. sp.	n. sp. + n. ssp.
New World									
Costa Rica	19	8	1	7	2	42.1	47.4	46.7	50.0
Cuba	150	61	10	1	2	40.7	47.3	1.6	4.1
Jamaica	74	40	13	?	?	54.1	71.6	?	?
Lesser Antilles	42	23		4		54.8	54.8	14.8	14.8
Average						*47.9*	*55.3*	*21.0*	*23.0*
Australasian–Pacific									
New Caledonia	31	14		3		45.2	45.2	17.6	17.6
NE-Australia	11+6	6+1		6-1		41.2	41.2	41.7	41.7
Hawaiian Islands	27	10	9	6	34	37.0	70.4	37.5	67.8
Gambier Islands	2	1		9		50.0	50.0	90.0	90.0
Average						*43.4*	*51.7*	*46.7*	*54.3*
Total Average						45.6	53.5	35.7	40.9
Central America	116	70	23			60.3	80.2	65.0	65.0

first true revision of the New Caledonian helicinids and also the first contribution based on a significant amount of material (460 lots from over 260 stations) collected throughout the islands mainly by Philippe Bouchet and Simon Tillier in the late 1970's and 1980's. Fourteen species were accepted out of 31 available names and only three species previously not recognized. One taxon was considered as misidentification of a species occurring somewhere else which is not surprising for an isolated island setting with endemic species.

An amazing conchological convergence of the two most widely spread species on Grande Terre and adjacent islands combined with high intraspecific variability constitute the main reasons for half of the synonyms that were created despite the inadequate recognition and description of consistent differentiating characters. Two of the new species are local endemics and were absent in all old collection material revised.

The study applied a conservative approach, but it is suspected that a few "species" may involve species complexes that could not be resolved with morphological methodology and the material available. Molecular analyses were impossible due to inadequate conservation of the collections.

Northeast Australia

Stanisic in Stanisic *et al.* (2010) described six new species from northeast Australia, thus, implying that this can be regarded as a revision although the presentation is very condensed within this huge opus. Six species were accepted out

of eleven previously described taxa listed by Stanisic *et al.* (2010). Another four species described from Australia (*suprafasciata* Sowerby, 1874, *zebriolata* Pfeiffer, 1865, *fulgurata* Cox, 1865, and *crassidens* Tate, 1899) were recognized as extralimital taxa by previous authors (*e.g.,* Iredale 1937). Stanisic *et al.* (2010) did not mention the obviously true East-Australian taxa *turbinella* Pfeiffer, 1855 and *yorkensis* Pfeiffer, 1862 which, have the potential to represent an older name for one of the new species from New South Wales (*turbinella*) and a synonym of *gouldiana* Forbes, 1851 (*yorkensis*). These six taxa were included in the count in table 1 in the outlined sense. Other Australian taxa occur outside the study area of Stanisic *et al.* (2010).

Hawaiian Islands

Neal (1934) provided a detailed analysis of the helicinids of the Hawaiian Islands based on extensive field collections and building on the previous, but less comprehensive study by Pilsbry and Cooke (1908). She accepted ten species and nine subspecies out of 27 taxa previously mentioned for the islands, six taxa were identified as erroneous records of species occurring elsewhere. Neal added six new species and 34 "varieties", adapting the idea of differentiating restricted local forms as was practice in other Hawaiian land snails groups with the most well-known genera *Achatinella* Reeve, 1850 and *Partulina* L. Pfeiffer, 1854. A formal, rather nomenclatural update by Richling (2011), including the study of types, presented a number of species and subspecific units in a similar range.

It is undoubted that the extreme isolation of the Hawaiian islands combined with a particularly rough topography and climate favored a high rate of endemism, but it remains to be investigated with modern approaches which taxonomic rank is appropriate or, applying terms of evolution, how much divergence the numerous subspecific units will show as such and in a larger frame. Initial molecular data were obtained from the very few surviving species by Leung *et al.* (2013) and the authors suspect cryptic species among the described varieties.

Gambier Islands

The most recent contribution on an island helicinid fauna revealed a previously largely unknown radiation on the small archipelago of the Gambier Islands located at the eastern outreaches of the Polynesian islands with a total diversity of ten species, of which only one was previously described and another species was erroneously reported for the archipelago (Richling and Bouchet 2013). This high diversity with up to eight species on a single island is outstanding for small Pacific islands, *e.g.,* the larger Henderson Island in the Pitcairn group was inhabited by only three species (Preece 1998).

Estimating diversity

The percentage of valid species in the eight studies analyzed ranged from 37 to 54.8% with an average of 45.6% (Table 1). When considering a splitting concept, which is here approached by accepting subspecies as units of diversity, this average climbs to 51.7% with a range from 41.2 to 71.6%. This means that about half of the number of taxa described or more fall into synonymy. On the other hand, after a rigorous revision (seven studies) 35.7% of the accepted taxa represent previously unknown diversity with only 1.6% in a well-studied area such as Cuba and up to 90% on the Gambier Islands. Applying the same splitting approach the newly described taxa constitute 40.9% with a range from 4.1% to 90%.

Thompson's (2011) catalogue of the Central American land and freshwater snails undoubtedly covers the largest area treated by a well-founded compilation including helicinids. Although it does not focus on revisions, it provides an estimate of diversity. Thompson accepted well-established synonyms and as a principle treated all taxa described below species level as subspecies. With these criteria he lists 70 valid species and 23 subspecies out of 116 available names (Table 1). His percentage of valid species is with 60–80% significantly above average, which is to be expected in a non-revisionary approach. For all land and freshwater snail families Thompson (2011) estimated that only about 35% of the diversity is known. He based his assessment on over half a century field experience throughout Central America, the diverse natural settings and the coverage in past explorations. Sixty-five percent of new taxa is a value clearly above the helicinid average and also higher than in Costa Rica which was once one of the most-poorly studied countries in Central America. If Thompson's estimate and the present considerations prove correct, helicinids would be among the better documented land snail families in Central America.

Comparing the number of valid taxa to the taxa described for a global estimate, 62% represent the "true" diversity in a rather conservative approach and 112% in the more splitting concept (Table 2). The Gambier Islands case, however,

Table 2. Recognized helicinid diversity after revisionary work for different areas compared to the number of available names and records for the area regardless of rank. For data sources see respective paragraphs. Numbers in parentheses also calculate the taxa from the Gambier Islands.

Area	Number of available names	In respective revision, number of valid taxa		Percentage of resulting recognized diversity	
		species	including subspecies	species	including subspecies
New World					
Costa Rica	19	15	18	78.9	94.7
Cuba	150	62	74	41.3	49.3
Jamaica	74	40+?	53+?		
Lesser Antilles	42	27	27	64.3	64.3
Average				*61.5*	*69.5*
Australasian–Pacific					
New Caledonia	31	17	17	54.8	54.8
NE-Australia	17	12	12	70.6	70.6
Hawaiian Islands	27	16	57	59.3	211.1
(Gambier Islands)	2	10	10	(500)	(500)
Average (incl. Gambier)				*61.6 (171.2)*	*112.1 (209.1)*
Total average (incl. Gambier)				61.5 (124.2)	90.8 (149.3)

appears abnormal in the small dataset. Considering the six other case studies are roughly representative for the whole area of distribution, the helicinid diversity would range from 770 to 1,140 species or up to 1,400 species if the studies from the Australasian-Pacific area are more typical.

Drawbacks in exploration

Helicinids meet a number of criteria that should make them favorites for researchers such as a size that makes them easy to work with, a comparatively high abundance among the larger land snail fauna in many areas, and—not to be ignored—their often colorful appearance. Despite these advantages and as shown above, even smaller local revisions of the family were not often attempted for the following possible reasons:

Questionable systematic concepts above species level

Until quite recently, higher systematics for the majority of helicinids, namely representatives of the genera *Helicina* Lamarck, 1799 and *Alcadia sensu* Wagner (1907–1911) and almost all Australasian and Pacific species, were based entirely on shell shape and opercular characteristics. The generic concept proposed by Wagner (1907–1911) was subsequently partially modified by seemingly applicable radular features (mainly Baker 1922a, but importance confirmed also by Bourne 1911), elaborated by the creation of new taxa and nomenclaturally corrected and adapted by the use of appropriate type species (Keen 1960), but essentially no other characters were incorporated. The major anatomical investigations by Bourne (1911) and Baker (1926, 1828) basically failed to find significant features and the value of radular characteristics was increasingly and rightfully questioned (Solem 1959, Boss and Jacobson 1973).

Thompson (1982) was the first to point to the importance of the embryonic shell structure and his detailed anatomical studies for the related families Proserpinidae and Proserpinellidae and systematic considerations greatly inspired subsequent research. Richling (2004a) evaluating characters of shell, radula, and anatomy for Central American taxa proved the applicability of features of the female reproductive system along with the embryonic shell structure as main distinguishing elements for Neotropical genera. Furthermore, doubts in radular features were confirmed and incongruity with Wagner's concept likewise revealed the shortcomings in opercular characteristics for the majority of genera. For more details and on the history of systematic concepts see Richling (2004a, 2009, 2011). The new hypotheses were further elaborated and applied to certain Pacific helicinids (Richling 2009, 2011), but a helicinid range-wide coverage as well as supporting molecular analyses are still in progress. Consequences for alpha-taxonomic work are further discussed in the next paragraph.

Limited recognized differentiating characters and convergence

While helicinids as family are clearly defined and easily recognized, differentiating shell characters on a lower systematic level are often missing. The majority of helicinids lacks the most directly accessible features like apertural barriers other than and because of the operculum, sophisticated breathing devices like other operculates, or obvious shell sculpture. Shell coloration and subtle differences in shell shape remain simple options. However both characters need to be used with great care and are often only meaningful when comprehensive collections of a given area and populations are available to evaluate the range of variation—a major weakness in many older descriptions. The operculum only rarely provides features for species delineation other than reflecting the shape of the aperture. Only some species of the genus *Eutrochatella* Fischer, 1885 in a very broad sense, of *Alcadia*, *Nesiocina superoperculata* Richling and Bouchet, 2013 (see also discussion in the species description) and the Stoastomatinae developed some unusual external structures on the operculum.

As in other snail families (*e.g.,* Helicidae, Orthalicidae, Bulimulidae, Achatinellidae), bright coloration is an adaptation to an arboreal lifestyle and, therefore, subject to multiple convergences. In helicinids, Richling (2004b) observed that the coloration in live snails can be realized in two different ways: through the "usual" shell coloration or, the colorful mantle pigmentation that shines through the thin, transparent shell. It is assumed that the latter is linked to habitats with limited calcium carbonate availability. Additionally, it is suspected that the visible coloration in some species changes throughout ontogeny with juveniles (and small sized species) being rather dark (perhaps imitating feces as camouflage) and the adults brightly colored and mottled.

Up to now comparative studies failed to find significant species-specific differences in the genital system (*e.g.,* Baker 1926, Richling 2004a, 2009) like those extensively described for several pulmonate families with little differentiation in external features and intergrading shell shapes (Lymnaeidae, Zonitidae, Oxychilidae, Succineidae, Hygromiidae, etc.). Therefore, shell shape still represents the most important morphological diagnostic feature on the level of alpha taxonomy for the Helicinidae. Circumstantial evidence suggests that, in fact, it constitutes a main driving force in helicinid speciation because more closely related species in a given area tend to diverge conchologically, *i.e.,* into different size classes, shapes, occasionally also connected with a different lifestyle like arboreal or ground-dwelling (Fig. 6 with examples for Costa Rican species of *Helicina* and New Caledonian species of *Sturanya* A. J. Wagner, 1905). However, independent evidence, *e.g.,* genetic characters, are still needed to avoid a circular argument. The only substantial molecular analysis of helicinids so far investigates geographically structured
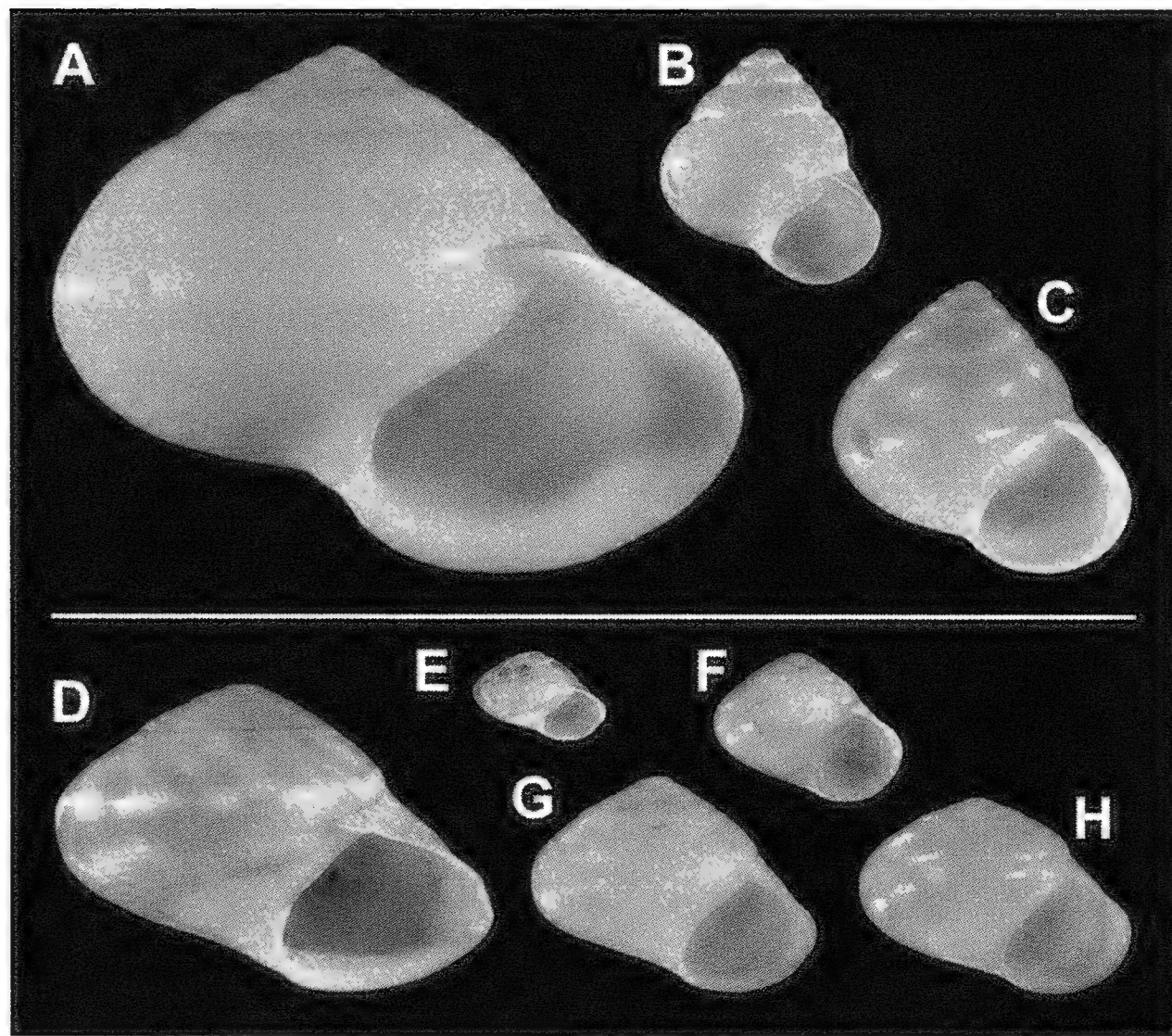
Figure 6. Typical co-occurrence of related helicinid species with different conchology. **Upper panel.** Species of the genus *Helicina* in the area of Río Barbilla, Costa Rica: **A,** *Helicina funcki,* **B,** *H. chiquitica* (Richling, 2001), **C,** *H. escondida* Richling, 2004, **Lower panel.** Species of the southern radiation of *Sturanya* on Mt. Mou, Grande Terre, New Caledonia: **D,** *Sturanya laeta* (Crosse, 1870), **E,** *S. littoralis* (Mountrouzier, 1859), **F,** *S. mouensis* (Crosse, 1870), **G,** *S. macgillivrayi* (L. Pfeiffer, 1855), **H,** *S. benigna* (Crosse, 1870) (ground-dwelling?). All at same scale.

subspecific taxa of *Viana regina* (Morelet, 1849) in Cuba. Preliminary results suggest that the conchological differentiation reflects phylogenetic relations rather than an ecophenotypic background (Herrera *et al.* 2013).

Most confusing patterns regarding shell shape occur when species of different phylogenetic entities are involved. Obviously, the evolutionary potential of the helicinid shell shape is rather limited, which is reflected in the observations above on limited characters, so that within different groups often similar morphologies have evolved. Against the background of partially still poorly understood higher systematics within the helicinids, this complicates alpha taxonomy. The most amazing example of such shell convergence I experienced was with the most widely spread and above mentioned New Caledonian species *Sturanya macgillivrayi* (L. Pfeiffer, 1855) and *S. novaecaledoniae* (Baird, 1873) which can only be reliably differentiated by microstructural differences of the teleoconch surface and postembryonic shell (Fig. 7), both features were found to characterize two also geographically structured radiations (Richling 2009). Nearly ten synonyms were created and no concept about the species' distribution existed before these characters were recognized.

Species complexes

The question of possible "cryptic" species within species complexes was brought up in the case study of the Lesser Antillean species, but equally applies to other areas with truly isolated populations of high conchological similarity usually combined with a high variability within the populations. The small, depressed, often angulated to carinated so-called "*Pleuropoma*"-species with a flame pattern that occur throughout parts of the southern Pacific (*e.g.,* on islands of New Caledonia, Vanuatu, Fiji, Samoa, Tonga, etc. encompassing taxa like *gallina* Gassies, 1870, *articulata* L. Pfeiffer, 1853, *vitiana* Mousson, 1870, *vitiensis* Mousson, 1865, *diminuta* Mousson, 1871, *subcarinata* Mousson, 1870) certainly represent a prime example. Adding to the conceptual difficulty, taxa were occasionally described with multiple type localities or with uncertain origin.

Here, traditional methodology approaches its limits and molecular case studies are urgently needed. Dispersal capability is one of the key questions and probably the least understood. However, this goes far beyond the difficulties in understanding specifically helicinid diversity and, thus, the scope of this paper.

Limited wet preserved material

As mentioned above, anatomical features revealed only limited applicability on species level differentiation, but proved invaluable for generic assignment (Richling 2004a, 2009) which is often impossible by shell characters alone. But even on species level simple information like the sex in this dioecious family can be substantial because of groups with a significant sexual dimorphism, *e.g.,* "*Gemma*"-group of the Costa Rican species (Richling 2004a). Radula features allow conclusions on the lifestyle (e.g., substrate preference) in absence of field observations. The need of ideally fresh and water-free preserved tissue samples for molecular analyses is obvious.

Personal observations show that only few museum collections have significant holdings of ethanol preserved helicinid material which is most likely explained by the tropical center of distribution. In early times there was no tradition for wet collections and in more recent times, growing legal restrictions seriously hamper diversity documentation and, thus, collections in the tropics. Furthermore, the habitat of this mostly forest-dwelling group is disappearing at dramatic rates rendering collections increasingly difficult to impossible when species already went extinct (see below).

Another practical drawback, if material was preserved, concerns the specific and deviating anatomy of helicinids when compared to other land snails. When bodies and shells were routinely separated to ensure better preservation especially in operculate species, important anatomical features were often irreversibly destroyed. In the majority
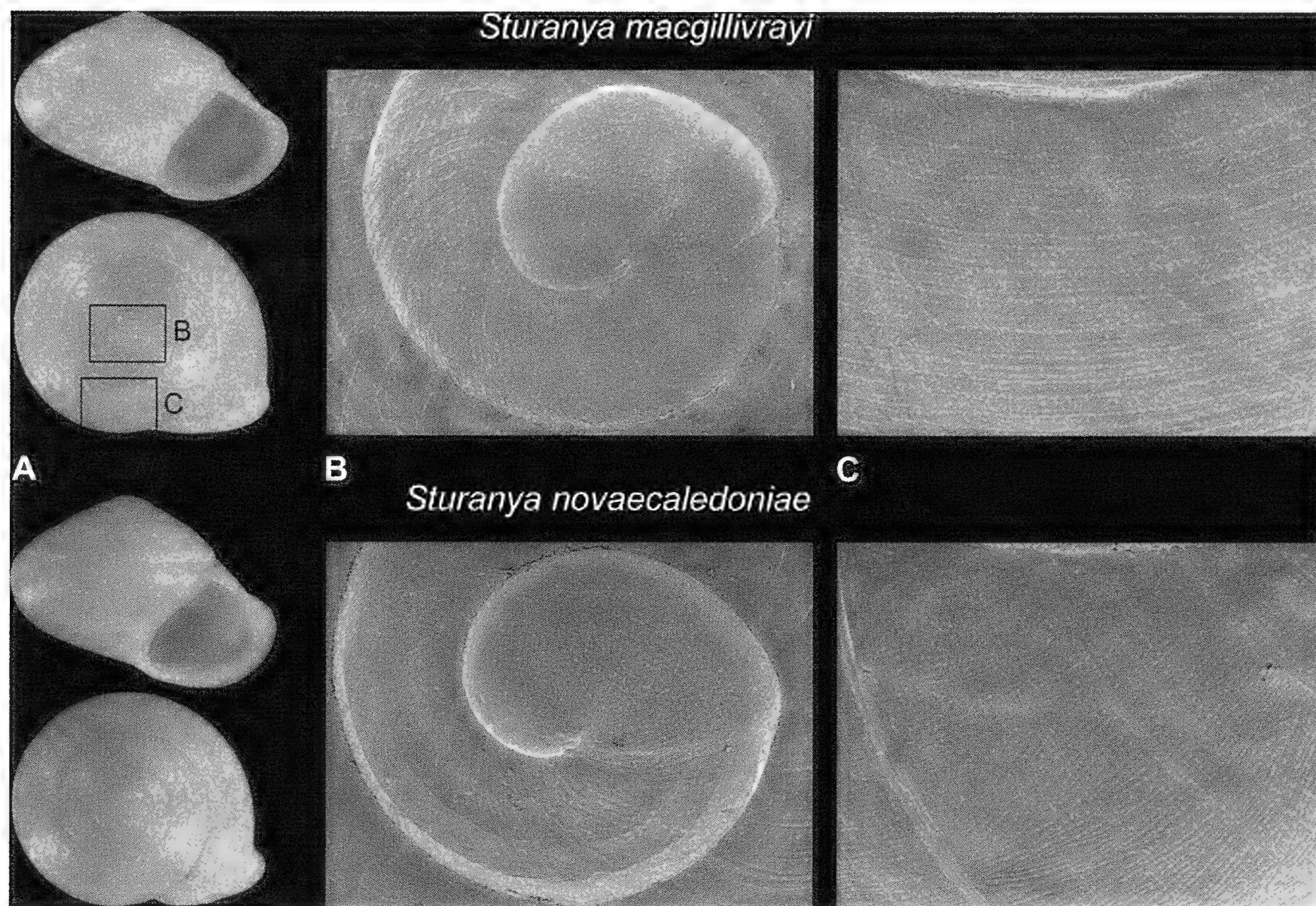
Figure 7. Convergence in the New Caledonian helicinids *Sturanya macgillivrayi* and *S. novae-caledoniae*, **A,** shell shape, **B,** surface structure on early postembryonic whorl with numerous fine parallel spiral lines in *S. macgillivrayi* and only 2–3 strong spiral ridges in *S. novaecaledoni-ae*, **C,** surface structure on later teleoconch with parallel lines in *S. macgillivrayi* and oblique diverging grooves in *S. novaecaledoniae*.

ranges. The knowledge of the habitat requirements of helicinids is rather limited to singular observations or general statements without detailed ecological analyses. These and personal observations indicate that most species are restricted to "wet" forest habitats. They range from coastal and moist to cloud forests with decreasing humidity as limiting factor of distribution. In Costa Rica Richling (2004a) observed that the most ecologically tolerant species, *Helicina tenuis* L. Pfeiffer, 1849, is absent in areas with dry forest (vegetation type after map by Tosi 1969) and less than 2,000 mm precipitation/year in regions with a marked dry season. In areas of less seasonally contrasting climatic conditions with higher humidity throughout the year the threshold of rainfall might be lower. In southern Veracruz, Mexico the same species was even observed in savannah forests (Baker 1922b). However, the ecological span of *H. tenuis* is rather outstanding and most other species have much more restricted distribution ranges and habitat requirements. While for Costa Rica Richling (2004a) reported several species also from secondary growth (rather shrubs) and even cultivated areas (*Helicina funcki* L. Pfeiffer, 1849, *H. pitalensis* A. J. Wagner, 1910, *H. beatrix beatrix* Angas, 1879, *H. b. confusa* (A. J. Wagner, 1908)), in these places remains of forests were still in the closer proximity or deforestation was not complete like in smaller hand-tended plantations under remaining trees (as found in remote, rural places of poor people). The other eight species of Costa Rican *Helicina* and *Alcadia* were so far only reported from natural forests.

In the middle of the last century massive deforestation started in the small Central American country with an original forest cover of about 90%. In 1984 Sader and Joyce (1988) calculated 17% remaining forest whereas a more recent analysis in 1991 with higher resolution resulted in 29%, but with increased fragmentation (Sánchez-Azofeifa *et al.* 2001). Tropical and premontane moist forest was almost completely eliminated by then and in vast areas only small disconnected patches were left over as remaining habitat for helicinids. The consequences for systematic studies are obvious: 1) field sampling can only result in equally or usually way more fragmentary records for a given species; 2) taxon/population sampling for different kinds of analyses will be rather chance driven than by scientific questions; 3) intergrading populations or sympatric occurrences of species—both important patterns

of pulmonates the distal part of the body and almost always the important part of the genitalia will be kept in good condition even if the body breaks whereas in helicinids the posterior pallial gonoduct with the systematically relevant and often very fragile structures will be ruptured and, thus, destroyed. The relevant parts would remain inside the shell and, thus, have been dried. The most frustrating example I have seen were different lots of *Sturanya uberta* (Gould, 1847) from O'ahu, Hawaiian Islands with numerous specimens in each lot but of which only 1–2% still possessed the required anatomical parts. These samples may represent the only preserved material of this extinct species and type species of *Orobophana* A. J. Wagner, 1905. Therefore, preserved helicinids should be kept with their shells when being prepared by people not familiar with the special body shape of helicinids. Finally, separate sexes cause another difficulty in that statistically twice the amount of material is required to be likely to have a female with the important structures.

Massive habitat loss, increasingly fragmented distribution, and extinction

While for a large share of helicinids, especially in the Australasian and Pacific region, populations/species are isolated *per se* by the colonization of or survival on archipelagos and islands, in other areas human impact fragments distribution

to understand species delineation—will often be erased; and 4) populations at type localities, especially of older descriptions, are often wiped out. A good example (with the exception of number 4) is given by *Helicina beatrix*, its subspecies, and their possible relation to *Helicina gemma* Preston, 1903 (for maps and details see Richling 2004a); molecular studies are still pending.

A much more dramatic picture is found on many Pacific islands. My own fieldwork on the 78 km²-sized Uvea island (belonging to the French overseas collectivity "Territory of the Wallis and Futuna Islands") in the Pacific during an invasive species survey (Meyer *et al.* 2008) recovered populations of two previously reported (Mousson 1871) species of helicinids. However both species very strictly limited to the very few remaining patches of native or fragmentary native forest on this strongly anthropogenically altered island. While the arboreal *Sturanya ueana* (Mousson, 1871) still occurred in seven of such isolated places, the second more ground associated species was found alive only in an area of less than one hectare. Although not as severely threatened as other tropical land snail families such as Endodontidae, Partulidae and Achatinellidae, the demise of helicinids on certain Pacific islands seems definite. A significant amount of taxa of the Hawaiian Islands obviously went extinct (Solem 1990, Leung *et al.* 2013) and only recently Richling and Bouchet (2013) described the helicinids of the Gambier Islands documenting the extinction of nine, mostly previously undescribed species representing 90% of the helicinid diversity. In this special case, the extinctions not only hampered our understanding of alpha-diversity by restricting the methodology to shell characters only, but at the same time on the generic level and for evolutionary processes, because the Gambier helicinids developed several shell characters unique for all Pacific species of the family. The current state of knowledge does not allow a well-founded generic assignment without an anatomical or potentially molecular analysis (Richling and Bouchet 2013).

ACKNOWLEDGMENTS

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Hypervariable or hyperdiverse, an independent assessment of the taxonomically confusing land snail genus *Tropidophora* (Pomatiidae: Littorinoidea: Caenogastropoda) in Madagascar[*]

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Abstract: The terrestrial littorinoid snail genus *Tropidophora* Troschel, 1847 is especially diverse on Madagascar with 94 currently recognized species and numerous named varieties. Supposed high within-species variation in shell and genital morphology led some researchers to suggest that these characters may be too variable to distinguish species, prompting them to conclude that the group may be taxonomically over-split. Recent sampling allows an independent test using COI sequence data which suggests that Madagascar's *Tropidophora* species are not over-named but are more diverse than previously thought. Molecular diversity is congruent with traditional morphology-based species concepts suggesting that future work using both molecular and morphological data sets will be useful in a taxonomic revision of the genus.

Key words: littorinoid, DNA barcoding, shell variation

Land snails are one of the most successful groups of terrestrial animals, second only to arthropods, with an estimated 35,000 to 64,000 species, only about 24,000 of which are described (Lydeard *et al.* 2004). Gastropods of at least eight major lineages have invaded land, some more than once (Rosenberg 1996). With no life stage capable of active long distance dispersal, land snails are notable for having high levels of geographic variation and genetically structured populations. This is especially true of larger species for which passive dispersal by wind, rafting, or transport on other animals is especially difficult. Land snail species are readily isolated and regional diversity can be quite high. Early surveys reporting high snail diversity in temperate habitats combined with anecdotal evidence of low diversity in tropical habitats led to the hypothesis that relatively cool, moist temperate areas with their associated deep leaf litter would have higher snail diversity than the tropics (Solem 1984), a pattern unlike most other plant and animal groups. However, recent surveys suggest that low abundance and incomplete sampling is responsible for this perceived pattern and that land snails in the tropics are often quite diverse (Schilthuizen 2011). The considerable field effort required to sample low abundance taxa has not only challenged macro-ecological studies but has also slowed species discovery and the taxonomic description of these species.

Perhaps the best documented tropical land-snail fauna is that of Madagascar, which was monographed by Fischer-Piette *et al.* (1993, 1994), who compiled all previously known taxa and described many additional species using the relatively meager collections available at that time. Between 1990 and 2009 Ken Emberton embarked on large-scale field studies, sampling approximately 1500 stations across the island. By 2003, 28 families, 77 genera and 685 species were reported from Madagascar (Pearce 2003). Later generic revisions (Emberton 2002a, 2002b, 2003a, 2003b, 2003c, 2003d, 2004, 2007) and field surveys (Emberton 2009, Emberton and Griffiths 2009a, 2009b, Emberton *et al.* 2010, Griffiths and Herbert 2013) brought the species total to approximately 1100, nearly equal to the 1200 species found in North America, north of Mexico, but in less than 3% of the land area. Even so, there are several groups collected in these surveys that have not been monographed, including one of the island's most diverse and taxonomically difficult groups, the pomatiid land snail genus *Tropidophora* Troschel, 1847.

Tropidophora are terrestrial littorinoid snails endemic to the east African coast and Indian Ocean islands of the Comoros, Mascarenes, and Seychelles where there are relatively few species, and Madagascar where the genus is particularly diverse. Madagascar's *Tropidophora* are found in eastern rainforests, northern and western deciduous forests and

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limestone *tsingys* (eroded limestone outcrops), and southern spiny thickets, but not in the open grassy and savannah habitats which often isolate these forests and outcrops. This patchwork of isolated habitats probably plays a role in the rich diversity of this and other groups in Madagascar (Quéméré *et al.* 2012).

The last treatment of Madagascar's *Tropidophora* (Fischer-Piette *et al.* 1993) recognized 94 species while treating 24 previously recognized species and 17 new forms as varieties of *Tropidophora tricarinata* (Müller, 1774) and seven species and one new form as varieties of *Tropidophora semidecussata* (Pfeiffer, 1847). These two species are highly variable in size, shape, color, and banding pattern and are distributed nearly island-wide, unlike other species of *Tropidophora*. Remarking on the need for independent data to evaluate the high level of shell variation within these and other *Tropidophora* species, Emberton (1995) developed a phylogenetic hypothesis for nine species from 40 populations using allozyme data. Comparing shell and genital characters from the same populations with the resulting cladogram, he concluded that high levels of within-species variation exist in both morphological data sets, thus these characters were too variable to unravel species level relationships and suggested that many more species and forms should be synonymized. However, the cladogram was poorly resolved and relied on few presumed synapomorphies to define species. Many of the populations treated as conspecifics in Emberton's analysis were considered different species by authors prior to Fischer-Piette's monograph. Therefore, if current species concepts are in fact complexes of multiple species, then high levels of "intra-specific" variation would be expected in the morphological data sets. It is still unclear if *Tropidophora* species are currently over-split because of high intraspecific variation as suggested by Emberton (1995) or if intraspecific variation appears remarkably high because of lumping of previously recognized species. Testing these alternative hypotheses requires independent data.

Emberton's field surveys collected 4400 specimen lots of *Tropidophora* from 1100 of his 1500 sampling sites, some live collected and alcohol preserved. This material was deposited at Florida Museum of Natural History at the University of Florida (UF) and specimens were selected to build a COI sequence library for terrestrial snails as part of a DNA Barcoding project to develop a species-level identification tool for land snails (Hebert *et al.* 2003). To date, 188 sequences have been generated for 60 nominal *Tropidophora* species. Here I use these data to test whether *Tropidophora* contains high levels of intraspecific shell variation or many unrecognized species. Rapidly describing the diversity of these forests is particularly important because Madagascar's high rate of deforestation has resulted in the loss of 40% of the islands forest cover since the 1950's (Harper *et al.* 2007), threatening species restricted to forested habitats with extinction.

MATERIALS AND METHODS

All 4400 specimen lots of *Tropidophora* collected in Emberton's surveys along with approximately 100 lots from other sources were sorted and specimens mature enough and in sufficiently good condition to identify were grouped into approximately 200 recognizable morphospecies based on shell characters. Each morphospecies was matched with a species or variety name using Fischer-Piette *et al.* (1993) or given a numerical designation if they could not be clearly matched. Sixty morphospecies were identified among the 188 specimens that were subsequently successfully sequenced. Of these, 29 could be assigned to *Tropidophora* species and eight to varieties of *Tropidophora tricarinata* recognized by Fischer-Piette (1993). The remaining 23 morphospecies included 16 clearly undescribed taxa and seven others which could not be unambiguously assigned to species or named variety without further taxonomic study and generic revision. Of the 23 numerically identified morphospecies, ten fit within the broad concept of *T. tricarinata* and would be considered varieties of that species based on Fischer-Piette's concept.

Approximately 2 mm³ pieces of foot tissue were cut from alcohol-preserved specimens, extracted with DNAzol (Molecular Research Center, Inc., Cincinnati), and a 658 bp fragment of the cytochrome c oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR) using primer pairs LCO-I490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) as described by Meyer (2003), and sequenced at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida. Other tissue samples were extracted, amplified by PCR and sequenced at the Canadian Centre for DNA Barcoding (CCDB), using their standard procedures. Voucher data, including images, georeferenced localities, and sequences are available from the Biodiversity of Terrestrial Snails (TSB) project stored at the Barcode of Life Data System (BOLD, www.barcodinglife.org) (Ratnasingham and Hebert 2007). Sequences were aligned by eye in Bioedit (Hall 1999). Nucleotide substitution models were calculated using hierarchical Likelihood Ratio Tests using ModelTest 3.7 (Posada and Crandall 1998) as implemented in PAUP (Swofford 2003). Two Bayesian analyses were conducted in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) using the general time reversible model with invariant sites and gamma distribution of rates across sites (GTR+I+G) based on ModelTest results. The two independent runs of 2 and 3 million generations were distributed over five chains, four heated and one cold. The temperature parameter was raised to 0.05. Trees were sampled every 1000 generations. Burn-in was 200 trees and run convergence was assessed by comparing the mean and variance of log likelihoods, both by eye and by examination of split frequencies. A maximum likelihood analysis using the

same model of evolution was performed using Randomized Axelerated Maximum Likelihood (Stamatakis *et al.* 2008). Intraspecific and interspecific genetic distances were calculated using a Kimura 2-Parameter model (K2P).

RESULTS AND DISCUSSION

All 60 morphospecies identified using shell characters were clearly separated in the COI trees from both Bayesian analyses (only one shown here, Fig. 1) and from the Maximum Likelihood analysis. The three analyses did not differ in the composition of terminal taxa but differed slightly in the position of several taxa and clades with low posterior probabilities (< 0.70) or bootstrap values (< 0.50). The 46 morphospecies represented by more than one sample were all reciprocally monophyletic, demonstrating that shell characters are not too variable within species to provide useful information in species delineation. In fact, morphological characters appear also to be useful in predicting species relationships, as shown by the clustering of morphologically similar taxa within well supported clades. For example, species in the clade composed of *Tropidophora* sp. 14, *T.* sp. 205, *T. virgata* (Sowerby, 1843) and *T. consocia* (Pfeiffer, 1848) all have high-spired straw-yellow shells with multiple dark bands and share similar habitats and geographic distributions in dry deciduous forests in far northern Madagascar. Similarly, the species in the clade composed of *T. aspera* (Potiez and Michaud, 1838), *T. thesauri* Fischer-Piette, 1949 and *T. moulinsii* (Grateloup, 1840) all have large, dark colored and banded, low-spired and strongly striated shells and inhabit northern deciduous forests. Even species within morphologically variable clades share unique characteristics. The clade that contains the largest species of *Tropidophora*, the strongly carinate *T. cuvieriana* (Petit de la Saussaye, 1841), also contains the relatively small lirate species *T. lirata* (Pfeiffer, 1852) and *T. denselirata* Fischer-Piette, Blanc and Salvat, 1969. All members of this clade (sp. 112–*T. virgo*) share a uniquely reflected and internally thickened peristome, a likely synapomorphy for the group, and are distributed in deciduous forests in northern Madagascar.

Even within the broadly defined *T. tricarinata* complex, several clades can be separated using shell morphology. Taxa in *T. tricarinata* clade 1 (Fig.2) inhabit northern rainforests and possess weakly striate but not carinate shells that are banded and have an orange-red stain on the columella that appears to be a synapomorphy for the group. The low-spired, smooth and striped taxa in *T. tricarinata* clade 5 are also morphologically similar and inhabit far south-eastern rainforests. Clades 2, 3, and 4 contain taxa that are restricted to north-eastern, central-eastern and south-eastern rainforests respectively,

but unlike the other *T. tricarinata* clades these taxa are morphologically quite variable. One of the most variable shell characters are the raised ridges for which the genus *Tropidophora* (keel bearer) is named and for which the species *T. tricarinata*, and its supposed varieties *T. bicarinata* (Sowerby, 1843) and *T. unicarinata* (Lamarck, 1822) are also named.

If most large *Tropidophora* from Madagascar's eastern rainforests are one highly polymorphic species as suggested by Fischer-Piette (1993), then molecular data should show that 'varieties' of *Tropidophora tricarinata* are monophyletic with respect to other *Tropidophora* species. Likewise, sympatric individuals of this species should be genetically similar because they have the opportunity to interbreed and are not reproductively isolated. However, if shell variation is not random and if taxa synonymized into, or named as varieties of *T. tricarinata* are in fact reproductively isolated species, then morphologically similar taxa should cluster into reciprocally monophyletic genetic groups that may occur sympatrically.

While traditionally-defined species were recovered as monophyletic groups in both the Bayesian and Maximum Likelihood analyses, the taxa synonymized into *Tropidophora tricarinata* form a paraphyletic assemblage at the base of the tree, so that a clade that includes all of them also includes all of the other sequenced *Tropidophora* species. Varieties in this complex were distributed among five major clades (Fig. 1, clades 1–5). Clade 3 also includes *T. zonata* (Petit de la Saussaye, 1850), a species not considered a variety of *T. tricarinata* by Fischer-Piette (1993). Two clades of other *Tropidophora* species, one composed of *T. magnei* Fischer-Piette, Blanc, Blanc and Salvat, 1993 and *T. vallorzi* Fischer-Piette, Blanc, Blanc and Salvat, 1993 and the other consisting of *T. pulchella* (Sowerby, 1843), *T.* sp. 31 and *T. perinetensis* Fischer-Piette and Bedoucha, 1965 form a polytomy with clade 3, nesting between clades 1, 2 and 4. Even if these species were to be synonymized with *T. tricarinata* the resulting 'species' would remain paraphyletic with respect to all remaining *Tropidophora*.

The 'varieties' of *Tropidophora tricarinata* are recognizable as morphospecies, are reciprocally monophyletic in COI, and have different geographic ranges. The taxa within each clade of *Tropidophora tricarinata* vary more in height/width ratio, presence and number of carinae, and color pattern than any other *Tropidophora* species. For example, clade 2 includes taxa with high-spired bicarinate and low-spired unicarinate shells. Clade 3 encompasses taxa with high-spired shells that are smooth or bicarinate and a low-spired taxon with a tricarinate shell. Likewise, clade 4 also includes low and high-spired forms. The other clades, while less variable in shell shape and sculpture, vary markedly in color. In most groups of snails, including other *Tropidophora* species, this level of morphological variation usually indicates multiple species and in fact many of these *Tropidophora tricarinata* varieties were originally described as species.
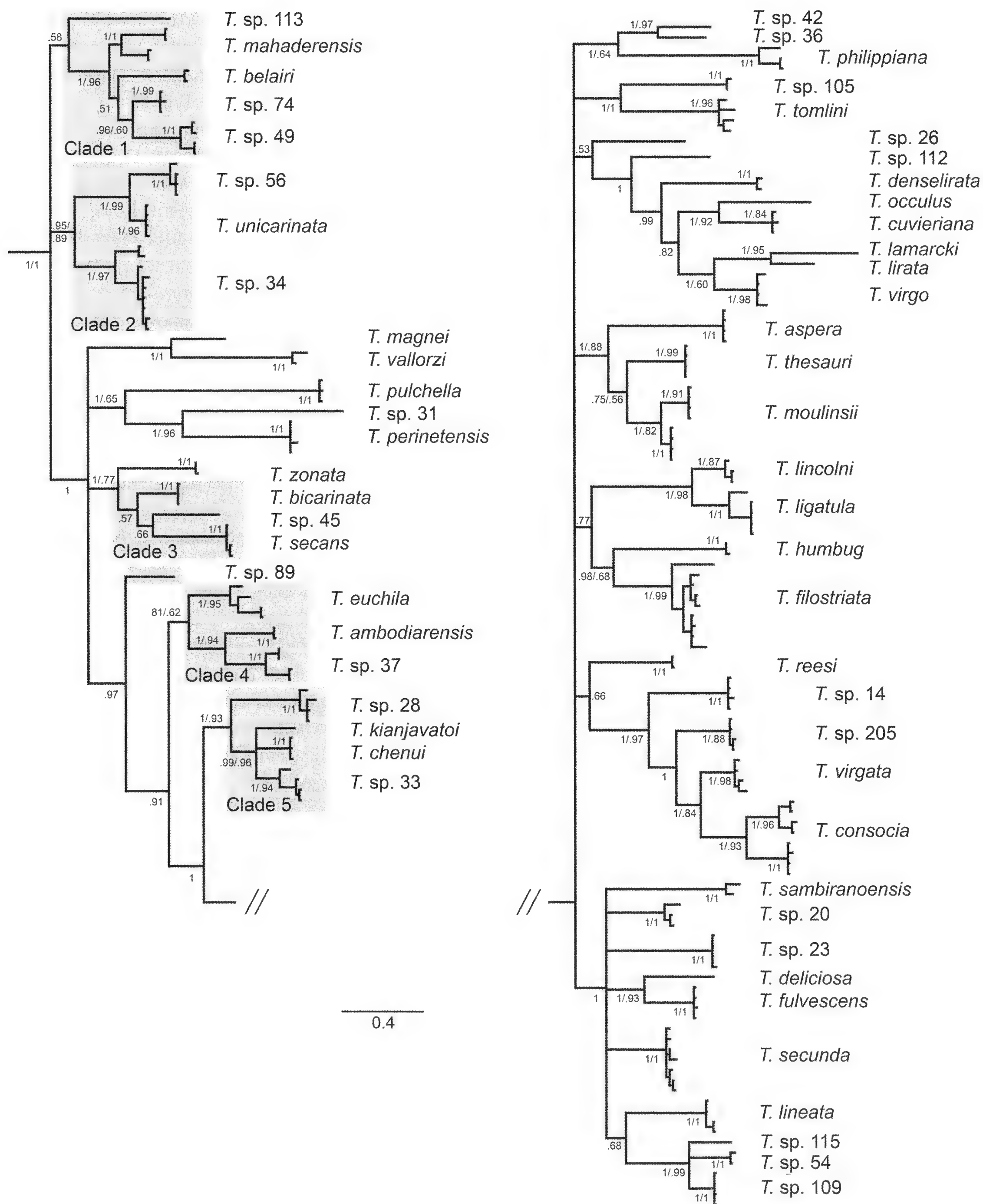
Figure 1. Majority rule Bayesian inference tree for Madagascan *Tropidophora* based on COI sequence data. Posterior probabilities (left) and Maximum Likelihood bootstrap values are given for nodes with greater than 50% support (right). Clades composed of *Tropidophora tricarinata* varieties are highlighted in gray. Branch lengths are measured in expected substitutions per site. Tree rooted with *Pomatias elegans* (Müller, 1774), not shown.

Intraspecific variation in COI sequence data is also particularly high within *Tropidophora tricarinata* as defined by Fischer-Piette (1993) especially when compared to older species concepts. Intraspecific variation in COI sequence data within traditionally defined species averages 1.97% (*SE* = 0.05%) while interspecific variation averages 12.06% (*SE* = 0.02%).
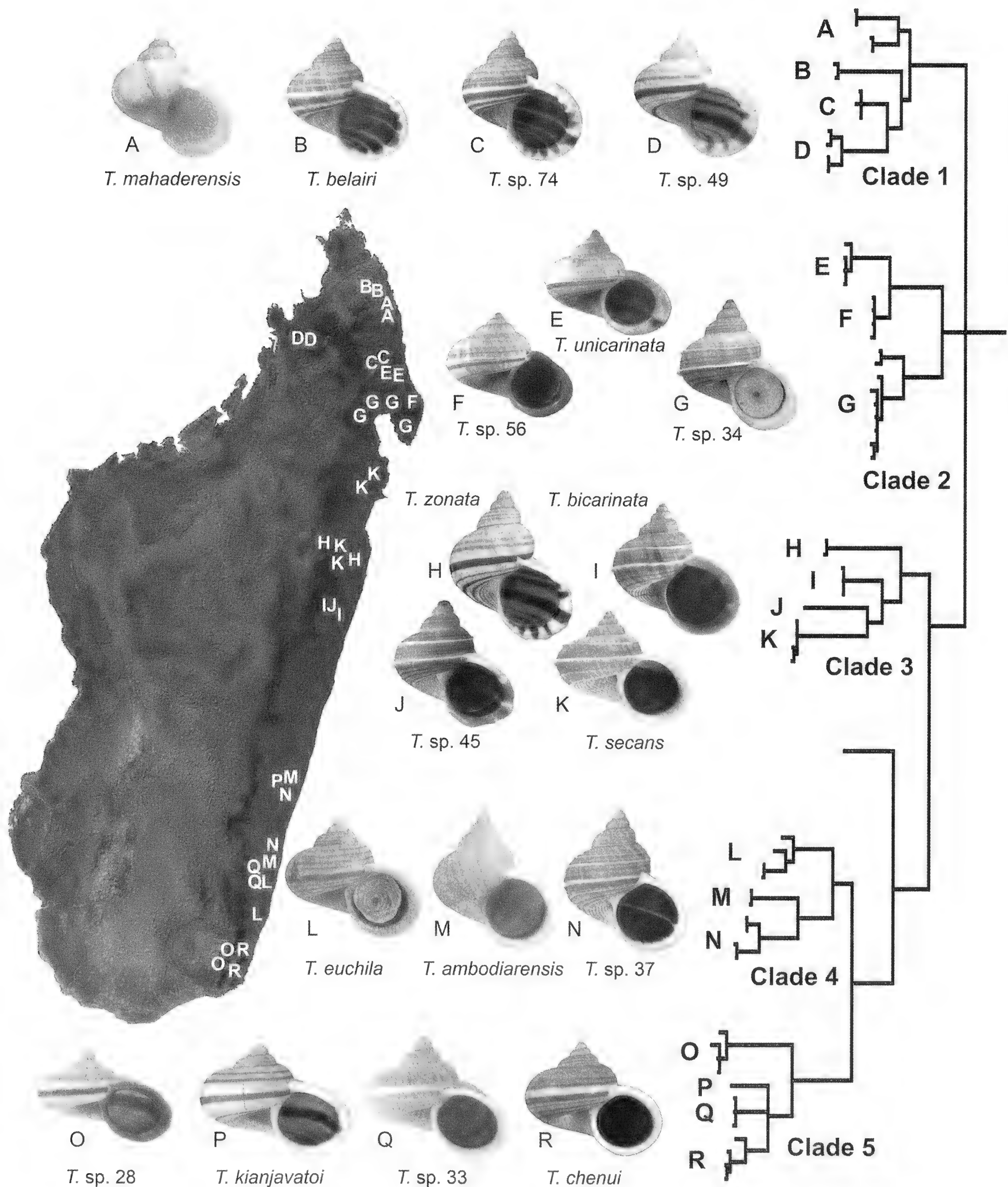
Figure 2. Clades of *Tropidophora* taxa attributed to *Tropidophora tricarinata* with their geographic distributions. (Color shown in electronic version only).

There is relatively little overlap in intraspecific and interspecific variation between 7–10% (Fig. 3). Conversely, intraspecific variation within the taxa synonymized by Fischer-Piette into *Tropidophora tricarinata* averages 10.04% ($SE = 0.06$%) and is bimodal, one peak falling within the intraspecific variation of the traditionally defined species and another peak largely overlapping interspecific variation in the genus.

Recent and extensive sampling by Emberton allows explicit tests of reproductive isolation between taxa considered varieties of *Tropidophora tricarinata* by Fischer-Piette (1993).
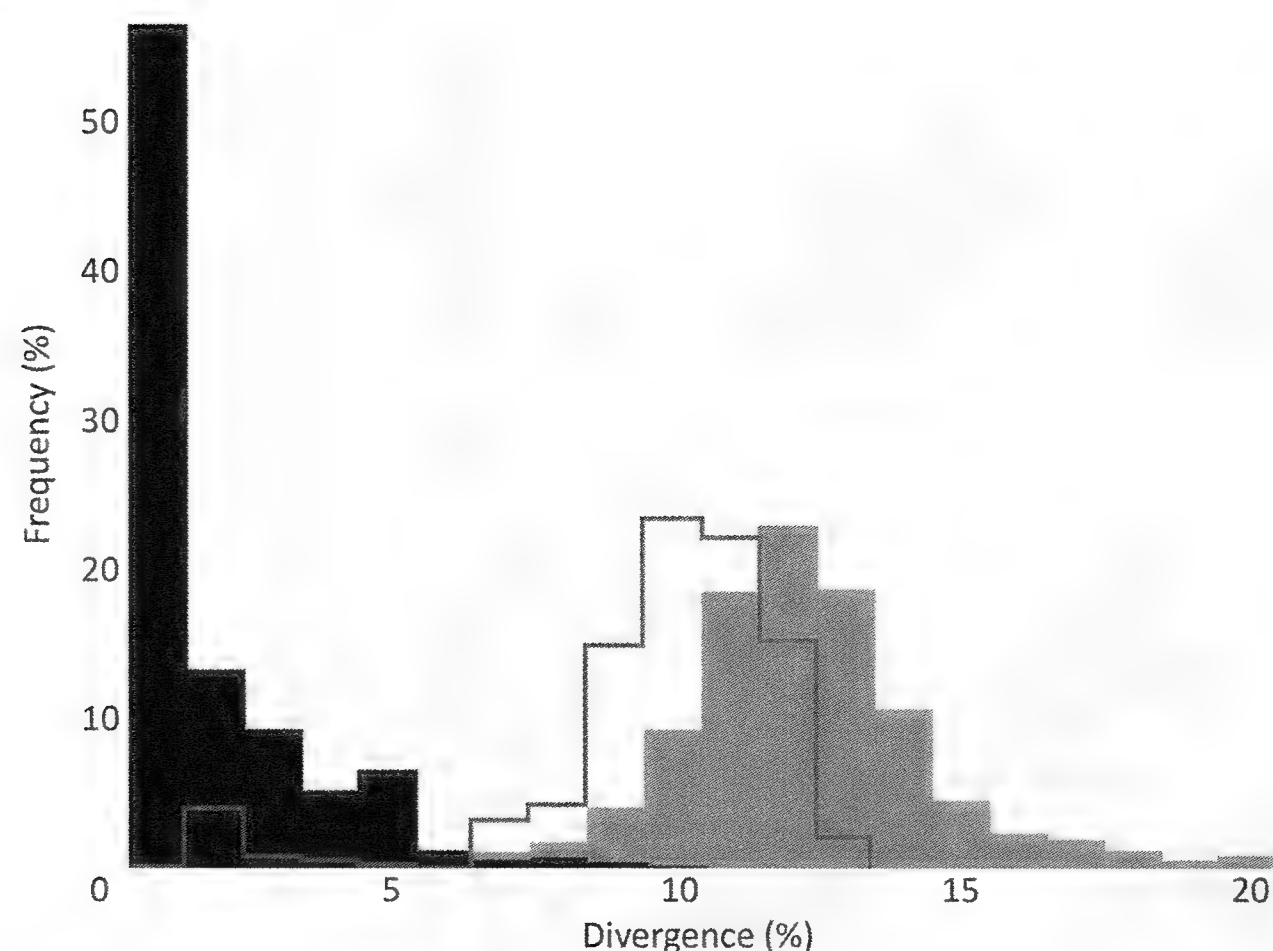
Figure 3. Distribution of normalized intraspecific (black) and interspecific divergences (solid gray) for *Tropidophora* based on species concepts prior to Fischer-Piette (1993) compared with intraspecific variation within *Tropidophora tricarinata* (gray outline) as envisioned by Fischer-Piette (1993).

Several pairs of taxa that would be attributed to varieties of *T. tricarinata* using Fischer-Piette's species concept occur in the same 20 x 20 meter study plots and were successfully sequenced. These include *T. bicarinata* and *T.* sp. 45 from Andriantanteley Massif, *T. secans* Fischer-Piette, 1949 and *T. zonata* in Zahamena Reserve; and *T.* sp. 37 and *T.* sp. 89 at Vatovavy Reserve. Each of these pairs have the opportunity to interbreed yet maintain unique shell characters and COI haplotypes, demonstrating genetic isolation, thus meeting the strictest definition of biological species. This is the most convincing evidence that *T. tricarinata* is a complex of different species and not one highly variable species. It is likely that the other named varieties of *T. tricarinata* that are not known to be sympatric but are equally divergent morphologically and genetically are also species level taxa.

Uncritically relying on a small group of characters to define species is likely to be deceptive in a diverse group like *Tropidophora*. Better sampling combined with independent sequence data demonstrate that some of the most visible shell characters used to group *Tropidophora tricarinata* varieties are taxonomically misleading, including the disposition of carinae for which the group was named. Superficially similar bicarinate shells *T.* sp. 34, *T. bicarinata*, *T.* sp. 45 and *T.* sp. 37 are found in three of the five clades of *T. tricarinata*, two of these are found microsympatrically. Bicarinate shells are also found in other *Tropidophora* species including *T. occlusa* (Mörch, 1852) and *T. cuvieriana* and this character is either symplesiomorphic or has evolved multiple times, and is, therefore, of limited phylogenetic utility. While the congruence

of morphologically defined taxa with COI sequence data shows that morphological characters are useful for defining species, it is also clear that some of these characters can be misleading.

Synonymizing multiple morphologically disparate species into *Tropidophora tricarinata* resulted in a 'taxon' that was morphologically highly variable. This confusing variation combined with limited fluid-preserved material for anatomical investigation or DNA analysis, resulted in the perception that the group was too challenging to revise (Verdcourt 1993). Congruence of COI data with morphologically-based species concepts suggests that COI sequence data is providing reliable information and that a revision of the group using a combination of morphological and genetic information is possible.

If nearly all species and named varieties of *Tropidophora tricarinata* are in fact species, then there are 143 described *Tropidophora* species in Madagascar, although those named as varieties after 1961 are not available names and will need to be re-established. Furthermore, there are approximately 60 additional morphospecies in collections that do not match any currently recognized species or named variety, which appear to be undescribed species-level taxa. This would bring the total number of species for the genus in Madagascar to approximately 200, more than doubling the number of currently recognized species. Modern taxonomic revision of the group combining shell and anatomical characters with molecular data will be needed to test these suppositions.

Madagascar's historically fragmented forest habitats have played an important role in the development of the islands diverse fauna (Quéméré *et al.* 2012). The land snail fauna is no exception; with more than 1100 species it is probably the most diverse mollusk fauna of any similarly sized terrestrial area. However, deforestation threatens these and other forest-adapted species with extinction. Most of Madagascar's conservation areas are chosen using data from surrogate species, usually vertebrates, rather than more diverse invertebrate groups like snails, despite the fact that snails and insects are better predictors of conservation priorities for vertebrates than the converse (Moritz *et al.* 2001). Unfortunately, poor taxonomy and until recently, sparse sampling have limited the use of snails in these conservation efforts. The availability of increasingly inexpensive sequence data is proving invaluable in rapidly uncovering species complexes, which can be efficiently targeted for taxonomic revision and detailed study. Unraveling the taxonomy of complex groups will uncover diversity that can be used to prioritize natural area conservation efforts as well as making these species accessible for evolutionary, ecological and other scientific investigation. These efforts are badly needed especially in species-rich and poorly-sampled tropical habitats that are rapidly undergoing profound habitat alteration.

ACKNOWLEDGMENTS

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Molluscan species diversity at North Pacific hydrothermal vents: What we know and what it may mean*

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Abstract: Hydrothermal vents in the deep sea are harsh, temporally unpredictable habitats with what appears to be a distinct fauna. Decades of subsea research with crewed and remote vehicles have generated a list of known species; is the species list complete? Evidence derived from mollusks sampled at hydrothermal vents on the East Pacific Rise (EPR), and Gorda and Juan de Fuca ridges suggests that the answer is yes. A 2006 compilation of hydrothermal vent species based on decades of research is updated and compared to specimens from these three active ridges in collections of the Field Museum of Natural History (FMNH) that resulted from limited collecting activity. Only three dives at each of the two Gorda Ridge vents collected all named species; 90% of the 20 species known from Juan de Fuca Ridge were collected in nine cruises. At the EPR, only 81% of the 43 known species were collected, but differences among the ridges were not significant. The limited FMNH collections increased the known ranges of six species from Juan de Fuca to Gorda Ridge and of nine species on the EPR. In addition, the EPR appears to host more rare species, potentially due to the frequent temporal changes at these vents. Mollusks currently known from each ridge, with their expanded ranges, are listed; the implications of these results for recent discoveries of slow-spreading vent fields are discussed.

Key words: Juan de Fuca Ridge; Gorda Ridge; East Pacific Rise; clam bed; collections

The deep sea, despite being the largest habitat on Earth, remains the least known. Since the discovery of seafloor hydrothermal vents on the East Pacific Rise, a large proportion of deep-sea research efforts have been devoted to these habitats. The rate of species description of the deep-sea hydrothermal vent fauna is unsurpassed. Has over 35 years of subsea and taxonomic research discovered all members of the vent fauna?

To address that question, I focus on the mollusk fauna of the East Pacific Rise (EPR) and the North East Pacific (NEP) Ridges of Juan de Fuca and Gorda. Mollusks offer an ideal group with which to address this question, as the phylum Mollusca comprises over 42% of the world's known vent fauna (Desbruyères *et al.* 2006); the Arthropoda and Annelida also form large percentages. The great depths at which vents lie, their distance from shore and the typically rocky terrain that surrounds them have limited prior collections of the vent fauna, meaning that few of the pertinent taxonomic groups had been discovered before the routine use of subsea vehicles. The onus of historical names that can create myriads of synonymies in more accessible faunas is thus avoided. Further, most vent mollusks from the North Pacific have been described by three experts (McLean 1988, 1989a, b,

1990, 1991, 1992, 1993, 2008, Warén and Bouchet 1986, 1989, 1993, 2001) who are uniquely familiar with the animals and who not only adhere to, but help establish, modern zoological standards. We can never answer the question of whether we know all species in a fauna, as one can never refute the existence of uncollected, undescribed species. However, the rate at which species are being described from these vents has declined and the list of known species is increasingly stable.

This paper documents the currently known molluscan species at hydrothermal vents on the East Pacific Rise (EPR) from 9° to 13°N and on the Gorda and Juan de Fuca Ridges (NEP). These areas are selected as they were the first hydrothermally active areas discovered, they have received considerable subsea research, and specimens from all three ridges are present in the collections of the Field Museum of Natural History. Are the mollusks that make up the hydrothermal vent fauna fully documented? How much collecting effort must be exerted to document the fauna at comparable, recently discovered vent fields?

The hydrothermal vent habitat

Hydrothermal vents release heat from deep within the Earth. As the heat warms the subsea crust, it initiates convection

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cells that draw seawater under the ocean floor. As the water transits the subsurface, it is heated and chemically reduced; oxygen is driven off and chemicals such as sulfates are reduced to sulfides. The hot, increasingly reduced fluid leaches metals from the sub-seafloor basalt and emerges supersaturated with metals. The metals precipitate, sometimes creating the illusion of smoke, when the hot fluid contacts the near 2° C seawater; large deposits of metal-rich sulfides can result (Van Dover 2000). Bacteria oxidize reduced chemicals to obtain energy; this energy in turn sustains the vent fauna. The existence of a bacteria-based ecosystem was unimagined before the discovery of hydrothermal vents in 1977 (Van Dover 2000).

Although the vent fluids contain energy-rich compounds that sustain microbial life, these same compounds can be toxic to animal life. High temperatures, low pH, high concentrations of dissolved heavy metals and deadly hydrogen sulfide in vent fluid may exclude non-specialized taxa from the vent habitat. In addition to its chemical rigors, the vent habitat is chronically unstable. Animal populations at vents can be affected by local and distant seismic events (Johnson *et al.* 2001); rock falls or events such as precipitation within a fluid conduit can alter or even stop fluid flow. Studies of fluid geochemistry have found that vent fluid salinity and often other characters change through the eruption cycle (Butterfield *et al.* 1997).

MATERIALS AND METHODS

The names and geographical ranges of molluscan species known from hydrothermal vents on the Gorda and Juan de Fuca Ridges (NEP) and the East Pacific Rise (EPR) reported by Desbruyères *et al.* (2006) were compiled; species descriptions or reports published more recently were added and are indicated in upper case in Tables 1 and 2. The widely available volume edited by Desbruyères *et al.* (2006) encompasses page-long genus or species-level treatments of the world's known vent fauna; authors are the appropriate experts for each taxonomic group. Although several years old, the treatments reflect decades of collecting and include photographs, diagnostic characters, biological information and the collection localities or known geographic ranges of each species. Are the decades of collecting effort that built this resource necessary to document the molluscan species at hydrothermal vents?

To address that question, the appended lists of mollusk species from NEP and EPR vents (Tables 1, 2) were compared to the mollusks collected from 1997 to 2003 during Remotely Operated Vehicle or the Human Occupied Vehicle ALVIN dives to vents on the three ridges. The collections were made during three dives in 2003 and 2004 at each of two active sites

on Gorda Ridge (and one collection from 1988), nine cruises to active sites on Juan de Fuca Ridge, and two three-week long cruises on the EPR. One EPR cruise focused on 9°N, an area rarely included in published accounts of the vent fauna (*e.g.*, Tunnicliffe and Fowler 1996, Desbruyères *et al.* 2006); some of the specimens from this cruise are not deposited at FMNH and were not included in this report. FMNH collections cover the EPR between 8°37′N, Stauromedusa Field (Voight 2006), and 13°N and the off-axis field, Caldera (Voight *et al.* 2004). The section of the EPR between 9 and 13°N has received the most subsea research, as gauged by the online ALVIN dive log (Woods Hole Oceanographic Institute 2012), and is the focal area for the EPR in this report.

The collections are deposited in the Field Museum of Natural History (FMNH), having been sorted and identified using species descriptions and other literature (with some material sent for expert verification). Solenogastres were not identified to species. Each lot has been entered into the online database of hydrothermal vent invertebrates (Field Museum of Natural History 2012).

The sampling effort required to fully document a fauna reflects the diversity of the fauna. If the FMNH collections, which result from comparative minor sampling effort, contain most, if not all, the mollusk species known after decades of research at these hydrothermal vents, these vent mollusks will be concluded to be well-documented. To test this statement, the number of mollusk species in all literature reports for each ridge was compared to the number of mollusk species in FMNH collections for that ridge. Assigning significance to differences in the number of taxa on each ridge remains somewhat problematic. If FMNH collections compare well with the list of known species, with at least 90% of the total number of mollusk species known, the fauna will be considered to have been well sampled in the years since the first biological cruise to the EPR in 1979, since the 1981 discovery of vents on Juan de Fuca Ridge and since the 1988 discovery of hydrothermal activity at Escanaba Trough, Gorda Ridge.

In addition, comparing FMNH records to published reports allows our knowledge of the fauna to be refined. New records of a species from beyond its published geographic range, *e.g.*, the presence of a species previously recorded only from 13°N at 9°N, constitutes a range extension. These are indicated by boldface in the appropriate table.

With so little known of background deep-sea species, distinguishing taxa closely associated with vents from accidental or opportunist taxa can be problematic. To minimize this, I exclude here species that are known to primarily occur in non-vent, non-chemosynthetic habitats. For example, octopuses of *Graneledone* Joubin, 1918 are routinely seen at clam beds at NEP vent fields (*e.g.*, Juniper *et al.*, 1992) but they range widely in the deep sea (Voight 2008) and are

Table 1. The 23 molluscan species known from hydrothermal vents on Gorda and Juan de Fuca Ridges are listed following Bouchet *et al.* (2005). Additions to the species ranges (reported following Desbruyères *et al.* 2006) are indicated for species in **Bold** with the range extension indicated after the taxonomic authority. UPPER CASE = addition to fauna. TLO = type locality only. Site abbreviations are: ET = Escanaba Trough, Gorda Ridge; GR-14 = Seacliff, Gorda Ridge; Axial = Axial Seamount, Juan de Fuca Ridge; ES = Endeavour Segment, Juan de Fuca Ridge; MV = Middle Valley, Juan de Fuca Ridge.

Family, taxon name, authority (new record)	Distribution from Desbruyères *et al.* (2006)
Gastropoda	
Neolepetopsidae, ***Neolepetopsis gordensis*** McLean, 1990. (GR-14, see also McLean 2008)	ET
Neolepetopsidae, *PARALEPETOPSIS TUNNICLIFFAE* McLean, 2008	MV, TLO
Fissurellidae, *CORNISEPTA VERENAE* McLean and Geiger, 1998	Axial, TLO
Pseudococculinidae, *AMPHIPLICA GORDENSIS* McLean, 1991. "from sulfide crust"	Gorda - ET
Pyropeltidae, *Pyropelta musaica* McLean and Haszprunar, 1987	Axial; whale fall (McLean 1992)
Lepetodrilidae, ***Clypeosectus curvus*** McLean, 1989b. (ET, GR-14)	JFR
Lepetodrilidae, *Lepetodrilus fucensis* McLean, 1988	JFR
Lepetodrilidae, *Lepetodrilus corrugatus* McLean, 1993	MV, TLO
Lepetodrilidae, *LEPETODRILUS GORDENSIS* Johnson *et al.*, 2006	Gorda
Sutilizonidae, *Sutilizona tunnicliffae* Warén and Bouchet, 2001	JFR - ES, TLO
Sutilizonidae, *Temnocinclis euripes* McLean, 1989b	JFR - ES
Neomphalidae, *Lacunoides vitreus* Warén and Bouchet, 2001	Axial, TLO
Neomphalidae, ***Melanodrymia brightae*** Warén and Bouchet, 1993. (ET)	JFR - ES
Peltospiridae, ***Depressigyra globulus*** Warén and Bouchet, 1989. (ET; GR-14)	JFR
Seguenzioidea (unassigned) *ADEUOMPHALUS TROCHANTER* Warén and Bouchet, 2001?	Co-Axial, TLO
Turbinidae, (Skeneinae) ***Fucaria striata*** Warén and Bouchet, 1993. (ET)	JFR - ES
Provannidae, ***Provanna variabilis*** Warén and Bouchet, 1986. (ET; GR-14)	JFR
Buccinidae, *BUCCINUM THERMOPHILUM* Harasewych and Kantor, 2002	JFR - ES, TLO?
Hyalogyrinidae, ***Hyalogyrina globularis*** Warén and Bouchet, 2001. (ET; MV)	JFR - ES
Bivalvia	
Vesicomyidae, ***Calyptogena starobogatovi*** Krylova and Sahling, 2006. (MV; Clam Bed)	Axial
Vesicomyidae, ***Calyptogena diagonalis*** Barry and Kochevar, 1999. (ET; MV)	Monterey Canyon
Vesicomyidae, ***Ectenagena extenta*** Krylova and Moskalev, 1996. (ET; MV)	Monterey Canyon
Solenogasters	
Simrothiellidae, *Helicoradomenia juani* Scheltema and Kuzirian, 1991	JFR; Gorda
Six species known only from one site; only from holotype unless otherwise noted.	
Neolepetopsidae, *Paralepetopsis tunnicliffae* McLean, 2008	MV One or two specimens
Fissurellidae, *Cornisepta verenae* McLean and Geiger, 1998	AXIAL Holotype; Tunnicliffe (2012) shows 3 specimens
Lepetodrilidae, *Lepetodrilus corrugatus* McLean, 1993	MV Holotype
Neomphalidae, *Lacunoides vitreus* Warén and Bouchet, 2001	AXIAL 1 collection of 11 specimens
Sutilizonidae, *Sutilizona tunnicliffae* Warén and Bouchet, 2001	Endeavour 2 specimens (Geiger 2012)
Seguenzioidea (unassigned) *Adeuomphalus trochanter* Warén and Bouchet, 2001	Co-AXIAL (2 specimens in running shoe caught in grab)

excluded. Species found at organic falls and vents (*e.g., Amphiplica gordensis* McLean, 1991; *Pyropelta musaica* McLean and Haszprunar, 1987) are included as members of the vent fauna, as are those also known from cold seeps (*e.g.,* vesicomyids *Ectenagena extenta* Krylova and Moskalev, 1996; *Calyptogena diagonalis* Barry and Kochevar, 1999). In their

description of *Adeuomphalus trochanter,* Warén and Bouchet (2001) noted that the two known specimens were collected from a running shoe recovered with a grab near a vent on Juan de Fuca Ridge; Kano *et al.* (2009) question whether other members of the genus (often associated with sponges) are vent-dependent. I include this species in the list with a question mark.

Table 2. The 43 molluscan species known from hydrothermal vents on the East Pacific Rise between 9° and 21°N are listed following Bouchet *et al.* (2005), with three yet to be described and eight species known only from inactive areas. Additions to the species ranges (reported following Desbruyères *et al.* 2006) are indicated for species in **Bold** with the range extension indicated after the taxonomic authority. UPPER CASE = addition to fauna. Gal = Galapagos. TLO = type locality only.

Family, taxon name, authority (updated distribution)	Distribution from Desbruyères *et al.* (2006)
Gastropoda	
Neolepetopsidae, *Eulepetopsis vitrea* McLean, 1990	21°N–17°S
Lepetodrilidae, ***Clypeosectus delectus*** McLean, 1989a (9°N; 11°25′N)	13°N; 21°N
Lepetodrilidae, ***Gorgoleptis patulus*** McLean, 1988 (9°N)	13°N; Gal
Lepetodrilidae, ***Gorgoleptis spiralis*** McLean, 1988 (9N; 11°25′N)	13°N
Lepetodrilidae, *Gorgoleptis emarginatus* McLean, 1988	9°–21°N
Lepetodrilidae, ***Lepetodrilus cristatus*** McLean, 1988 (9°N)	13°N; 21°N
Lepetodrilidae, *Lepetodrilus elevatus* McLean, 1988	21°N–17°S
Lepetodrilidae, *Lepetodrilus pustulosus* McLean, 1988	21°N–17°S
Lepetodrilidae, *Lepetodrilus ovalis* McLean, 1988	21°N–17°S
Lepetodrilidae, *Lepetodrilus galriftensis* McLean, 1988	9°N; Gal
Lepetodrilidae, *Lepetodrilus tevnianus* McLean, 1993 (see Johnson *et al.* 2008; Bayer *et al.* 2011)	11°N
Sutilizonidae, *Temnozaga parilis* McLean, 1989a	21°–13°N
Neomphalidae, *Cyathermia naticoides* Warén and Bouchet, 1989	9°–21°N
Neomphalidae, *Melanodrymia aurantiaca* Hickman, 1984	21°N–17°S
Neomphalidae, *Melanodrymia galeronae* Warén and Bouchet, 2001	13°N, TLO
Neomphalidae, *Neomphalus fretterae* McLean, 1981	9°–21°N
Neomphalidae, *Pachydermia laevis* Warén and Bouchet, 1989	21°N–17°S
Neomphalidae, *Planorbidella planispira* Warén and Bouchet, 1989	21°N–17°S
Neomphalidae, *Solutigyra reticulata* Warén and Bouchet, 1989	13°N; 21°N
Peltospiridae, ***Ctenopelta porifera*** Warén and Bouchet, 1993 (9°N)	13°N
Peltospiridae, *Echinopelta fistulosa* McLean, 1989a	13°N; 21°N
Peltospiridae, *Hirtopelta hirta* McLean, 1989a	13°–21°N
Peltospiridae, *Lirapex granularis* Warén and Bouchet, 1989	9°–21°N
Peltospiridae, *Lirapex humata* Warén and Bouchet, 1989	21°N, TLO
Peltospiridae, ***Nodopelta heminoda*** McLean, 1989a (9°N)	13°; 21°N
Peltospiridae, ***Nodopelta rigneae*** Warén and Bouchet, 2001 (9°N)	13°N
Peltospiridae, ***Nodopelta subnoda*** McLean, 1989a (9°N)	13°N
Peltospiridae, *Peltospira delicata* Warén and Bouchet, 1989	9°–13°N
Peltospiridae, *Peltospira lamellifera* Warén and Bouchet, 1989	13°N, TLO
Peltospiridae, *Peltospira operculata* McLean, 1989a	9°–21°N; 17°S
Peltospiridae, *Rhyncopelta concentrica* McLean, 1989a	21°N–17°S
Chilodontidae, ***Bathymargarites symplector*** Warén and Bouchet, 1989 (9°N)	13°N; 21°N
Provannidae, *Provanna ios* Warén and Bouchet, 1986 (9°N; 11°25′N)	17°S; 13°–21°N
Provannidae, *Provanna muricata* Warén and Bouchet, 1986	21°N; Gal
Conidae, *Phymorhynchis major* Warén and Bouchet, 2001	13°–9°N
Bivalvia *n* = 3	
Vesicomyidae, *Calyptogena magnifica* Boss and Turner, 1980	21°N–23°S
Mytilidae, *Bathymodiolus thermophilus* Kenk and Wilson, 1985	13°N–21°S
Pectinidae, *Bathypecten vulcani* Schein-Fatton, 1985	9°–13°N
Solenogastres *n* = 4	
Simrothiellidae, *Sensilloherpia pholidota* Salvini-Plowen, 2008	13°N, TLO
Simrothiellidae, *Diptyaloherpia insolita* Salvini-Plowen, 2008	13°N, TLO
Simrothiellidae, *Helicoradomenia acredema* Scheltema, 2000	21°N; Gal; 17°S
Simrothiellidae, *Helicoradomenia bisquama* Scheltema, 2000	21°N, TLO

Table 2. (Continued)

Family, taxon name, authority (updated distribution)	Distribution from Desbruyères *et al.* (2006)
Cephalopoda *n* = 1	
Octopodidae, *Muusoctopus hydrothermalis* (González and Guerra, 1998) (9°N, 13°N; 11°25′N sight record)	13°N; 23°S
To be described	
Lepetodrilidae, *Lepetodrilus* aff. *galriftensis* McLean, 1988 see Johnson *et al.* (2008)	
Seguenzioidea (unassigned family) *Moelleriopsis* sp. Bush, 1897; Warén and Bouchet 1989	
Conidae, *Gymnobela* sp. A. Warén and Bouchet, 2001	13°–9°N
Eight species from inactive areas or vent margins; data from species descriptions	
Neolepetopsidae, *Neolepetopsis verruca* McLean, 1990	Inactive chimney at 21°N
Neolepetopsidae, *Neolepetopsis occulta* McLean, 1990	Sulfide at Green Seamount 21°N
Neolepetopsidae, *Neolepetopsis densata* McLean, 1990	Inactive chimney at 13°N
Fissurellidae, *CLATHROSEPTA DEPRESSA* McLean and Geiger, 1998	Volcano 5, 1160 m depth near 13°N, TLO
Fissurellidae, *CORNISEPTA LEVINAE* McLean and Geiger, 1998	Summit volcano 6, 1775 m depth near 13°N.TLO
Sutilizonidae, *Sutilizona theca* Warén and Bouchet, 2001	Inactive sulfides, *n* = 3, 13°N
Anatomidae, *ANATOMA JANETAE* Geiger, 2006	Caldera, 13°N; 8° 37′N
Anatomidae, *ANATOMA BATHYPACIFICA* (Geiger and McLean, 2010)	13°N, TLO (with anemones and serpulids)

Because the rate of species description is predicted to slow as a fauna becomes better known, rate changes can indicate our knowledge of the fauna. The publication dates for all mollusk species from each of the three ridges were compiled and the median calculated. In addition, the number of species described in 2000 (or since) were totaled for each ridge. A pulse of recent species descriptions would indicate recent discovery, with all things (such as delays in preparing the description) being equal.

Rare and/or narrowly endemic species could indicate insufficient sampling, areas of high endemism, or they could indicate suspect inclusions which limit our knowledge of true diversity (Thessen *et al.* 2012). Thus, species which available data indicate occur at only one site and those known only from the type series merit special attention. To consider whether high endemicity at a site might relate to other factors, the site's depth and basic geology were considered. In addition, changes in vent fluid chemistry over the eruption cycle could affect faunal composition. To consider whether the collection of rare species relates to the timing of rare events, such as eruptions, I relied on literature accounts (Johnson *et al.* 2008, Bayer *et al.* 2011) that, although they do not report the deposition of voucher specimens, include specimen photos and cite singular events.

To document how molluscan species diversity compares among different vent fields on a ridge, the number of different species recorded from each vent field that was sampled at least nine times was compared. All samples, from push cores to grab samples of tube worm clusters with all associated fauna, were included. Including only fields that are represented by at least nine samples attempts to assure comparable sampling effort. Within Juan de Fuca, the total number of species collected from Endeavour Segment, a large complex area (Kelly *et al.* 2012), was determined. All samples from a ridge were pooled to determine the total sampling effort for that ridge and the total number of molluscan species collected from that ridge.

RESULTS

Table 1 lists 23 mollusks including 19 gastropods, three bivalves, and a solenogastre known from hydrothermal vents on Gorda and Juan de Fuca Ridges in the Northeast Pacific. Eight species have been added to those species listed for Juan de Fuca and Gorda Ridges by Desbruyères *et al.* (2006), including 3 species described in or after 2006. Two vesicomyid species described from cold seeps near Monterey Canyon are recorded on both ridges. Gorda Ridge hosts 12 known species, including two vesicomyids. FMNH records extend the known ranges of six species known from Juan de Fuca to Gorda Ridge (Table 1). In total, 10 or 43.5% of all species range more widely than reported in Desbruyères *et al.* (2006).

Juan de Fuca Ridge is home to 20 known molluscan species, including two described from FMNH collections. Hydrothermal vents on Axial Volcano host three species that are not known to occur elsewhere on the ridge. Only the type

collections of two (*Cornisepta verenae* McLean and Geiger, 1998; *Lacunoides vitreus* Warén and Bouchet, 2001) are known (Table 1); the third species, *Pyropelta musaica*, is also known from whale falls (McLean 1992). Middle Valley, Juan de Fuca Ridge, a sedimented hydrothermal field, has the highest known molluscan species diversity of these vent fields with widely distributed Juan de Fuca Ridge species and two (*Lepetodrilus corrugatus* McLean, 1993; *Paralepetopsis tunnicliffae* McLean, 2008) known only from one or two specimens (Table 1). Clam Bed on Endeavour Segment has 12 species.

The description of the limpet, *Lepetodrilus gordensis* Johnson, Young, Jones, Warén and Vrijenhoek, 2006, documents it to be endemic to Gorda Ridge; although the species has been collected from Juan de Fuca Ridge, this collection was concluded to be contamination (Voight *et al.* 2012). *Amphiplica gordensis*, described from a sulfide crust at Escanaba Trough, has been reported from GR-14 by Clague *et al.* (2001) and genetically identical specimens have been collected from wood and whale falls (Kano *et al.* 2013). Clague *et al.* (2001) also report *Provanna laevis* Warén and Ponder, 1991, described from Guaymas vents in the Gulf of California and present at North Pacific cold seeps (Sahling *et al.* 2002), from GR-14. Given the difficulties in distinguishing North Pacific species in *Provanna* Dall, 1918 (A. Warén, pers. comm.), I choose to leave this question unresolved.

Table 2 lists the 43 mollusk species known from EPR vents from 9° to 21°N, with what may be three additional vent-associated species as yet unnamed. In addition are the eight species (Table 2) described from either inactive areas such as extinct sulfides or from near vents but among what appear to be typically background fauna, *i.e.*, *Anatoma bathypacifica* (Geiger and McLean, 2010) and *A. janetae* Geiger, 2006. I do not discuss these in detail. FMNH collections, most notably from vents near 9°N and Flo and Bucky at 11.415°N, expand the known distribution of nine species (Table 2) that had been known only from 13°N. The range of nearly 21% of the species is expanded here compared to that reported in Desbruyères *et al.* (2006). Two species, (*Provanna ios* Warén and Bouchet, 1986; *Muusoctopus hydrothermalis* (González and Guerra, in González *et al.*, 1998)) that had been known only from sites far to the south and to the north, were recorded from 9°N. Their distributions are likely more continuous than has been documented.

Three gastropod species described from EPR vents, *Melanodrymia galeronae* Warén and Bouchet, 2001, *Lirapex humata* Warén and Bouchet, 2001, and *Peltospira lamellifera* Warén and Bouchet, 1989, remain known only from their type localities, all either at 13° or 21°N. These and three solenogastres known only from their type locality composed nearly 14% of the mollusk fauna. As *Provanna muricata* Warén and Bouchet, 1986 has been collected from 21°N and from near the equator at the Galapagos vents (Desbruyères

et al. 2006), it would seem likely to also occur between 9° and 13°N. *Lepetodrilus tevnianus* McLean, 1993 has been reported in the taxonomic literature only from near 11°N (Warén *et al.* 2006), but as discussed below, Johnson *et al.* (2008) and Bayer *et al.* (2011) report that it occurs more broadly.

The median years of species description, 1989 for the EPR and 1993 for both Juan de Fuca and Gorda Ridges, are not significantly different. Neither do the number of species descriptions published in or since 2000 differ among the ridges (Gorda, 1; Juan de Fuca, 7; EPR 9; G-test; $G = 1.988$).

FMNH collections hold all molluscan species known to occur on Gorda Ridge, despite having specimens from just three dives to each of the two known vent fields. Of the 20 mollusk species known to occur on Juan de Fuca Ridge, 90% were collected in 9 cruises involving FMNH. Only 35 of the 43 known mollusks (81%) from EPR vents are present in FMNH collections made during two three-week long cruises, although not all the material collected during these cruises was deposited at FMNH. However, no significant difference in the proportion of known species that were collected exists among the three ridges (G-test; $G = 0.1895$, $df = 5$).

Ridge membership appears strongly associated with species diversity; the EPR has the most (45) molluscan species and Gorda Ridge has the least (12). Within a ridge, more sampling at a vent field usually increases the total species collected (Table 3). Exceptionally, more species were documented in fewer samples from Clam Bed on Juan de Fuca Ridge than from Main Field Endeavour. The presence of a clam bed habitat may increase the total species.

DISCUSSION

Collections at FMNH hold 100% of the mollusks known from Gorda Ridge, and 90% and 85% of those known from Juan de Fuca Ridge and the East Pacific Rise, respectively. The high proportion of the known species collected from active vents during two cruises to Gorda Ridge, nine to Juan de Fuca Ridge and two to the EPR suggest that this limited collecting effort effectively sampled the mollusks known at these hydrothermal vents. The molluscan species level diversity at these active ridges is well known.

These collections also resulted in specimens that served as the basis for the descriptions of two vent-associated species in the NEP (*Hyalogyrina globularis* Warén and Bouchet, 2001, *Lacunoides vitreus*) and two species collected with non-vent fauna on the EPR (*Anatoma janetae*, *A. bathypacifica*). Does the description of 10% of the Northeast Pacific molluscan vent species from these collections contradict this statement? Specimens of *H. globularis* had been collected as early as 1988 (FMNH 309720). Its discovery was likely delayed by its overall similarity to the peltospirid, *Depressigyra globulus* Warén

Table 3. The number of discrete sampling events and the cumulative number of species in FMNH collections from each ridge, by site and pooled.

	Sampling Events	Mollusk spp. Collected	Spp. Known from Ridge
Gorda Ridge			
Escanaba Trough	21	12*	
GR-14 (Seacliff)	9	6	
Total Gorda Ridge	**30**	**12***	12
Juan de Fuca Ridge			
Main Field Endeavour	10	8	
Clam Bed	9	12*	
Endeavour Segment (Main Field Endeavour, Clam Bed, Raven, Quebec, Beach, Axial)	40	16*	
Total Juan de Fuca Ridge (Endeavour Segment above plus Middle Valley)	**44**	**18* (90%)**	20
East Pacific Rise			
9°N	26	29*	
13°N	19	20	
Caldera (slightly off-axis)	23	19	
13°N + Caldera	42	30	
Total East Pacific Rise (Stauromedusa Field (8°37′N); 9°N; Flo and Bucky (11°25′N); 13°N; Caldera)	**80**	**35* (81%)**	43

* Indicates a clam bed was sampled.

and Bouchet, 1989. The species *L. vitreus*, discussed further below, may represent one of the vanishingly rare species that may exist undetected in most habitats.

At both NEP and EPR vents, six species are known only from their type localities. Given that more species occur on the EPR, in terms of percent, this is nearly twice as many. Nine species from each area are also identified as having range extensions, percentage-wise, nearly twice as many at NEP vents than at EPR vents. Does this indicate the northern fauna is less well known? I suspect that the difference in range extensions reflects the uneven research effort at Juan de Fuca and Gorda Ridges. Gorda is notorious for poor weather; it has been subject to less research than has the more northern ridge. Whether the different frequencies of species known only from their type localities are biologically meaningful, I leave open to discussion, while noting that nearly half of those at EPR in this category are solenogastres, which are rarely identified to species, while those at NEP are all gastropods.

The harsh, temporally dynamic and stochastically distributed vent habitat is often dominated by one or very few species. The perpetual problem in determining the true species diversity at vents can be locating rare taxa among the hyper-abundant ones. For example, Juniper *et al.* (1992) report three gastropods, *Lepetodrilus fucensis* McLean, 1988, *Depressigyra globulus*, and *Provanna variabilis* Warén and Bouchet, 1986 and two polychaetes, *Ridgeia piscesae* and

Amphisamytha galapagensis, to comprise over 73% of all animals in 8 samples from Middle Valley, a vent with high molluscan diversity. Northeast Pacific limpets of *L. fucensis* and *L. gordensis* host bacteria on their gills (Bates 2007). Perhaps reliance on symbiotic bacteria frees the limpets from competition for food; they can live in stacks and reach extraordinary densities (> 100,000 m^{-2}; Bates 2007).

Not included here with the vent fauna are species from inactive sulfides (Table 2), most of which are known only from their type localities, or only from the holotype. These areas of previous hydrothermal activity are the target of proposed subsea mining, yet are among the least known vent-associated habitats. Whether these species are obligate associates of this habitat, how common they are, how they feed, and whether they exploit a special niche created by these habitats remain unanswered questions.

Two sites, Axial Volcano and Middle Valley appear to have unusually high numbers of endemic species (Table 1). On the Mid-Atlantic Ridge, the fauna of the two shallowest vent fields, Lucky Strike and Menez Gwen, is slightly more diverse than that of the deeper vent fields; depth may be a causal factor in this difference (Van Dover *et al.* 1996, Van Dover 2000). Depth may also contribute to the presence of unique species at Axial, the shallowest vent field on Juan de Fuca Ridge. Middle Valley is unusual in being sediment covered, as is Escanaba Trough on Gorda Ridge. These two vent fields, separated by 2500 km, share two species, *Ectenagena*

extenta and *Solemya* Lamarck, 1818 sp. (represented only by shell fragments from the hydrocarbon seep) that are otherwise unknown from Northeast Pacific Ridges. At a hydrothermally modified hydrocarbon seep at Escanaba Trough on Gorda Ridge, the bivalves *Ectenagena extenta*, *Solemya* sp. and *Calyptogena diagonalis* Barry and Kochevar, 1999 use hydrocarbons for energy, but they can only be sustained due to hydrothermal input (Kvenvolden *et al.* 1986, Clague *et al.* 2001).

Differences in ridge spreading rate appear to be linked to differences in ridge species diversity (Van Dover 2000). EPR has the fastest spreading rate, which has been linked to smaller, less stable vents, and higher species diversity. At EPR vents, phases of faunal succession characterize the initiation and denouement of hydrothermal activity. Tube worms of *Tevnia* typically colonize newly active vent sites, to be succeeded by tube worms of *Riftia*, which in time are replaced by mussels of *Bathymodiolus thermophilus* Kenk and Wilson, 1985 (Hessler *et al.* 1988, Mullineaux *et al.* 2000). Comparable successional changes likely also affect smaller taxa. One example may be *Lepetodrilus tevnianus*, a species so rare in collections that Warén *et al.* (2006) considered it to be known only from its type locality at 11°N. However, Bayer *et al.* (2011) consider it and *Ctenopelta porifera* Warén and Bouchet, 1993, to be "pioneer" species. They collected over 2600 individuals at post-eruption vents at 9°N; Johnson *et al.* (2008) also report it from 9°N to 7°S.

A comparable sequence of faunal change at NEP vents is as yet unknown (Tsumuri and Tunnicliffe 2001, Marcus *et al.* 2009), but *Lacunoides vitreus*, to my knowledge collected only once, may be an example. The 11 specimens were collected 6 months before the 1998 eruption of Axial Volcano in the midst of a massive new settlement of the vent gastropod species. Resolving what species remain undiscovered at hydrothermal vents requires that collections be made throughout the eruption cycle. Although unpredictable, the cycle is likely much longer on slow spreading than on fast-spreading ridges. Even on the best-studied ridges, currently unknown taxa may characterize some stages, despite the significant effort that has already been expended.

At EPR vents, a snapshot view of the fauna would show that gastropods partition habitats to a much greater extent than they do on NEP vents. The hot smoker habitat supports species of *Nodopelta* McLean, 1989a and the tube worm habitat supports species of *Lepetodrilus* McLean, 1988; at NEP vents, the smoker habitats are largely mollusk-free and *L. fucensis* or *L. gordensis*, *Provanna variabilis* and *Depressigyra globulus* partition the remaining habitat by temperature (Bates *et al.* 2005).

The existence of cryptic species, or species complexes, is still being unraveled among the vent fauna (*e.g.*, Stiller *et al.* 2013). Their presence cannot be refuted among the vent mollusks. Johnson *et al.* (2006) used gene sequences to distinguish *L. fucensis* from *L. gordensis* and supported the genetic difference with morphological differences. Although this discovery could be cited to argue that the formerly contiguous Gorda and Juan de Fuca faunas have begun to diverge to a greater extent than Table 1 indicates, these *Lepetodrilus* spp. may be exceptional. Both *L. fucensis* and *L. gordensis* rely on symbiotic bacteria that are thought to oxidize sulfide in the vent fluids for food (Bates 2007). Differences in the concentration of sulfide in the vent fluids of the two ridges, with that of Gorda being more limited (Von Damm *et al.* 2005, 2006), may have accelerated divergence of these species (Beinart *et al.* 2012).

Implications for the future

Ridge spreading rate has been associated with the size and stability of vent fields and the number of active vents, with slow spreading ridges having few, large and stable vents (Baker and Hammond 1992, but see Baker and German 2004 for a different view). If the slow spreading rate of Gorda Ridge is linked to its low species diversity, it bodes well for current research. Recently discovered vents in the first phases of exploration lie on slow-spreading ridges, such as those in the Indian Ocean, the Arctic and the Cayman Trough (Connelly *et al.* 2012, Nakamura *et al.* 2012, Rogers *et al.* 2012, Tao *et al.* 2012). Time-tested collection methods may efficiently and rapidly document most of the fauna of vents on these slow-spreading ridges.

The most recent reports of new vents also report the discovery of new species, but do not name the new species. The basis of those statements is that the COI gene sequence of these new species differs sufficiently from that known for other, previously named congeneric taxa (Connelly *et al.* 2012, Nakamura *et al.* 2012, Rogers *et al.* 2012, Tao *et al.* 2012). Whether such analyses represent the new minimum for species determination will depend on whether specimens of the new taxa are shared with expert taxonomists. Experts who describe species not only identify how species differ and assign names, they provide fundamental information on morphology and anatomy. These collections can be used to build robust phylogenies that will allow us to understand not just species, but their evolution and the history of hydrothermal vents.

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Cypraeidae: How well-inventoried is the best-known seashell family? *

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Abstract: Cowries (Cypraeidae) are diverse and occur in all tropical and some temperate seas, from shallow waters to the deep sea. There is a vast literature documenting the distribution, taxonomy, and shell variation, among many other topics. Because of its popularity among shell collectors, amateurs have contributed more to the cowrie literature than to most molluscan families. Traditionally, taxa have been described based on shell characters, and the majority of species descriptions continue to be published in non-peer-reviewed journals, magazines and books. Molecular studies in the last two decades have largely confirmed phylogenies based on morphology and anatomy. Currently, 245 species and 166 subspecies (or 411 operational taxonomic units) are recognized in 48 genera in the family. Despite being the best-known seashell family, the discovery curve suggests that there are still more taxa to be discovered. A review of the knowledge of the family is presented, and information on each genus is summarized.

Key words: morphological and molecular characters, inventory, species discovery curve, WoRMS, synonyms

Cowries (Caenogastropoda: Cypraeidae) are arguably the best-known seashells (Taylor 1979). Their polished and often colorful shells have attracted the attention of humans for thousands of years, and have been used for decoration, as currency, and in religious rituals. Cowries are very popular among shell collectors and most shell collections have at least some cowries; Dance (1966) suggested that shell collecting started with cowries. They are found worldwide; most species live in shallow and warm waters and share similar distribution with hard corals (Taylor 1979), but a few species live in temperate waters.

The goal of this paper is to review the literature, summarize the knowledge on cowrie diversity, and document the progress of its inventory.

The Cypraeidae have a rich literature spanning from scientific to popular, covering a wide gamut of topics. There are perhaps more monographs and books dedicated to the Cypraeidae than to any other molluscan family (*e.g.*, Gray 1824, Jousseaume 1884, Roberts 1885, Melvill 1888, Hidalgo 1906–1907, Schilder and Schilder 1938–1939, 1971, Allan 1956, Burgess 1970, 1985, Walls 1979, Liltved 1989, Lorenz and Hubert 1993, 2000, Lorenz 2001, 2002, Wilson and Clarkson 2004); there is even a book dedicated to a single species: a study of the Hawaiian endemic *Cribrarula gaskoini* (Reeve, 1846) (Dayle and Heiman 2011).

Several iconographies illustrate well the diversity in the family (*e.g.*, Sowerby 1870, Burgess 1970, 1985, Walls 1979, Lorenz and Hubert 1993, 2000, Poppe 2008); some also illustrate live animals (*e.g.*, Johnson 2008–2013). Most books on seashells from tropical regions include cowries. Several works focus on regional faunas (*e.g.*, Hawaii: Schilder 1933, Ingram 1937, 1947, Kay 1961, Cate 1965; Australia: Iredale 1935, 1939, Lorenz 2001, Darrah 2002, Wilson and Clarkson 2004; New Caledonia: Dautzenberg 1952, Chatenay 1977; Fiji: Cernohorsky 1964; Philippines: Cate 1966; Okinawa: Cate 1967; Mauritius: Schilder 1960a, Boizot *et al.* 1985; South Africa: Liltved 1989; Red Sea: Heiman 2000). *The Cowry* was a small journal dedicated to cowries published between 1960–1965 (R. J. Griffiths, ed.). A colorful new magazine, *Beautifulcowries*, has recently been launched as a new outlet solely for cowrie articles (Passamonti 2012).

Among the topics discussed in cowrie publications are: distribution (*e.g.*, Schilder and Schilder 1938–1939, Schilder 1965, 1969, Lorenz and Hubert 1993, 2000); geographic variation (*e.g.*, Schilder and Schilder 1938–1939, Irie 2006); and species complexes (*e.g.*, Lorenz 2001, 2009, Moretzsohn 2003, Lorenz and Chiapponi 2005). Many articles concern the description of new taxa (*e.g.*, Burgess 1993, Lorenz 2002, Moretzsohn 2002a, 2007); taxonomic revisions of genera or species complexes are less common

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(*e.g.*, Darragh 2002, Lorenz and Chiapponi 2005). The classification of the Cypraeidae has been a topic of discussion (*e.g.*, Schilder and Schilder 1938–1939, 1971, Schilder 1941, Steadman and Cotton 1946, Kay 1957, 1960a, Meyer 2003, 2004). In response to increasing splitting of genera, which reached 61 (Steadman and Cotton 1946), Kay (1957) proposed classifying all cowries in the single genus *Cypraea* Linnaeus, 1758. Several authors followed her advice (*e.g.*, Burgess 1970, 1985, Walls 1979, Liltved 1989), while others called groups of species either species complexes, subgenera or genera (*e.g.*, Schilder and Schilder 1971, Lorenz and Hubert 1993, Meyer 2003, 2004). Shell collectors tend to prefer the simplicity of a single genus, but scientists and more specialized collectors use a classification based on phylogenetic relationships.

Traditionally, cowrie classification has been based primarily on shell characters, despite the plasticity of such characters and the lack of shell sculpture and a visible protoconch in the adult shell. A few authors have used anatomical characters (*e.g.*, mantle: Burgess 1983; radula: Bradner and Kay 1995, 1996, Lorenz 1998, Moretzsohn 2002a, 2002b, 2004), or sought novel taxonomic characters (*e.g.*, protoconch: Ranson 1967, Foin 1982; odontophores, shell spotting, and dorsal line angle: Moretzsohn 2002b, 2003). Molecular characters have been used in the past two decades with the advent of molecular systematics and improvements in DNA sequencing technology; the effort was championed by Meyer (2003, 2004, 2005).

In contrast, studies of the animal (other than the shell) have received less attention, but there are some good studies on: biology and life history (*e.g.*, Taylor 1979, Kay 1985, Katoh 1989, Wilson 1998, Wilson and Clarkson 2004); ecology (*e.g.*, Hayes 1983, Lorenz 1998, 2000); anatomy (*e.g.*, Schilder 1936, Kay 1957, 1960b, Gosliner and Liltved 1985, Simone 2004); mantle (*e.g.*, Thompson 1961, Burgess 1983, Kay 1985); sperm (Healy 1986); reproduction (Ostergaard 1950, Taylor 1975, Wilson 1985, Osório *et al.* 1999, Meyer 2003, Wilson and Clarkson 2005); behavior (*e.g.*, Ostergaard 1950, Renaud 1976), etc. Other topics that appear less frequently include evolution (*e.g.*, Kay 1996, Meyer 2003, 2004, 2005), phylogeny (*e.g.*, Schilder 1936, 1939, Ranson 1967, Meyer 2003, 2004, Lopez Soriano 2006), and fossils (Beddome 1898, Schilder 1932, 1935, Kay 1990, Darrah 2002, Groves 2010).

Intraspecific variation has been discussed at length (shell morphometrics: *e.g.*, Schilder and Schilder 1934, 1952, Orr 1959, Schilder 1960b, Tissot 1984, Moretzsohn 2002a, Heiman 2004, 2005, Dayle and Heiman 2011). Sexual dimorphism in shell size has been documented in several species (*e.g.*, Orr 1959, Griffiths 1961, Schilder and Schilder 1961, Moretzsohn 2002a, Irie and Adams 2007, Irie and Morimoto 2008). Among the species studied, females usually tend to have a larger shell than males, but in a few species the opposite is true (Schilder and Schilder 1962). Sexual dimorphism has also

been reported in the radula and odontophores (*Cribrarula gaskoini*: Moretzsohn 2003); more studies are needed to verify if the same also occurs in other species that show dimorphism in shell size.

There are six major biogeographical regions where cowries are found ("cowrie provinces"): 1) Indo-Pacific; 2) southern Australian; 3) South African; 4) Mediterranean and West African; 5) Caribbean; and 6) West American. The Indo-Pacific is by far the largest and richest province, and includes the most widely-distributed species, as well as many with restricted ranges. The majority of cowries possess planktonic veliger larvae that can have long larval duration and good dispersal ability, which accounts for some widely-distributed species in the Indo-Pacific. In contrast, in a few regions with upwelling and/or high latitude, direct development has evolved independently at least five times (Meyer 2003). The following genera have crawl-away juveniles: *Cypraeovula* Gray, 1824 in southern Africa, *Barycypraea* Schilder, 1927 in southern Africa and Oman; *Austrocypraea* Cossmann, 1903, *Notocypraea* Schilder, 1927, *Umbilia* Jousseaume, 1884, and *Zoila* Jousseaume, 1884 in southern Australia; and *Muracypraea* Woodring, 1957 in northern Colombia and Venezuela. The latter genus occurs in low latitude but in upwelling areas (Meyer 2003).

MATERIALS AND METHODS

The list of valid species and subspecies used in this analysis was downloaded from the WoRMS database (WoRMS Editorial Board 2013) on 24 February 2013. Although WoRMS was created in 2008 as an outgrowth of the European Register of Marine Species, some entries in the Cypraeidae date back to at least 1997. I became the first taxonomic editor of the Cypraeidae in December 2010. Since then, I have attempted to clean up, complete the listing of all valid taxa, and add sources (citations), but to date little effort has been made to enter synonyms.

The distribution of cowries is well known and populations are reasonably well documented, therefore, subspecies are commonly recognized in the cowrie literature. Additionally, there are many more named taxa such as varieties and forms that are used by shell dealers and the shelling community, but those names are not governed by the International Code of Zoological Nomenclature; generally infrasubspecific taxa are not included in WoRMS and, therefore, not included in this analysis.

The distribution range maps (Fig. 1) of 209 Indo-Pacific species were traced into ArcGIS 8.3 based primarily on Burgess (1985), Lorenz and Hubert (2000), and Lorenz (2002), and complemented with vetted museum records. Figure 1 was modified from a work on Indo-Pacific biodiversity (Moretzsohn and McShane 2004); hence only species from that region were included (with a few extralimital cowries).
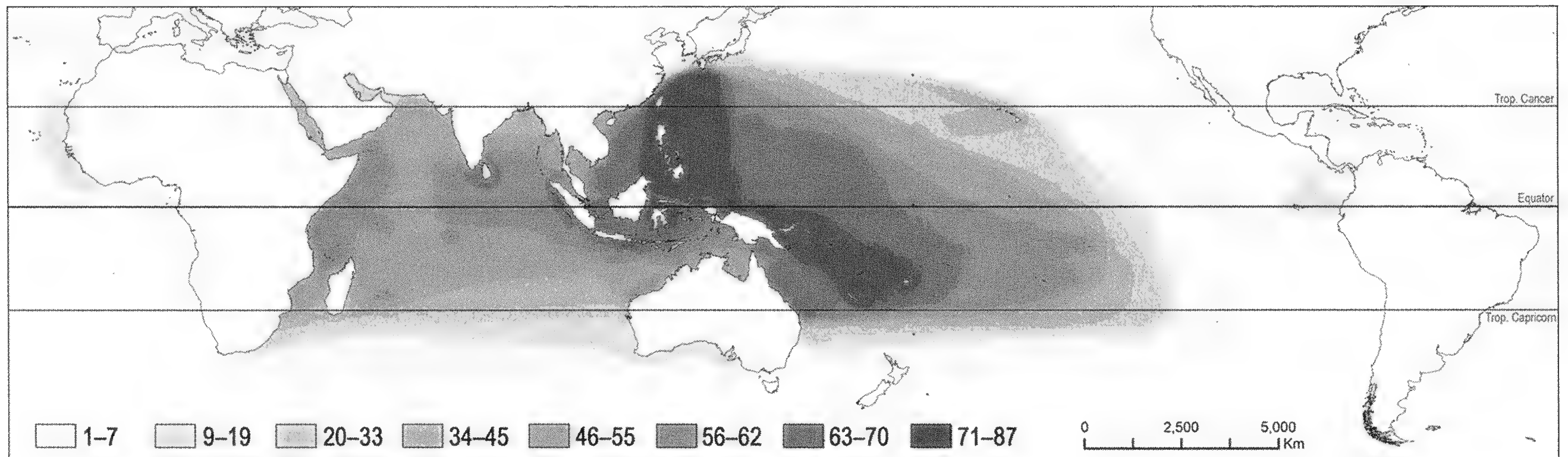
Figure 1. Pattern of diversity based on the distribution of 209 Indo-Pacific cowries. Figures represent species richness (modified from Moretzsohn and McShane 2004). (Color shown in electronic version only).

The Cypraeidae taxon list was downloaded from WoRMS in MS Excel format. Then, the following criteria for operational taxonomic units (OTU) (modified from Meyer 2004) were applied: 1) geographic distinction or allopatry; 2) taxonomic recognition by previous workers; and, 3) significant genetic distance or anatomical difference from sister group. An OTU was included in analyses only if at least two of the above criteria were met; alternate representation taxa, and fossil species were removed from the analysis.

Histograms of number of taxon descriptions were calculated using the online Free Statistical Software (Wessa 2012) and imported into MS Excel; pie charts and species discovery curves were produced in MS Excel 2010. Shell length and bathymetric ranges were obtained mainly from Burgess (1985), Lorenz and Hubert (1993), and Lorenz (2002), in addition to other sources; OTUs with incomplete shell size or depth data were excluded. Modal sizes and depths were determined from a database containing all available data compiled for all OTUs.

Authors' country of origin were obtained mainly from Coan *et al.* 2012, and several other sources; individual co-authors were accounted for in the pie charts of authors and countries (Fig. 3A, B), hence the number of entries is larger than the number of taxa. Note that although Franz A. Schilder was born in Austria, he grew up and worked in Germany and is often referred to as German, thus he was coded here as German.

For Appendix 1, authorities of genera and type species were found in Schilder and Schilder (1971), Lorenz and Hubert (2000), and Meyer (2003). The number of OTUs per genus was obtained from WoRMS (Moretzsohn and Gofas 2013). Shell size range, bathymetric range and distribution represent the extremes for each entire genus. Information on direct developers was found in Meyer (2003).

RESULTS

As of 24 February 2013 there were a total of 245 species and 166 subspecies recognized as valid in the Cypraeidae (Moretzsohn and Gofas 2013), or 411 OTUs (Appendix 1). The number of genera currently accepted is 48, with an average of 8.6 OTUs per genus. Seven genera are monotypic, and ten genera have 15 or more OTUs.

Most cowries are found in the tropics but a few species live in temperate waters in latitudes as high as 44°N (Savona, Italy) and about 42°S (Tasmania, Australia) (Beddome 1898). There is a latitudinal gradient in species diversity, with species richness increasing toward the tropics (Fig. 1), where cowries often share similar distribution with hard corals and live in shallow waters (Taylor 1979). The highest diversity of cowries is found in the Indo-West Pacific (IWP), in the region known as Coral Triangle, an area encompassing most of Indonesia, Malaysia, the Philippines, Papua New Guinea, the Solomon Islands, East Timor, and Brunei, with 71 to 87 species observed (out of 209). The region extending to Vanuatu, Fiji, and New Caledonia is also very diverse (Moretzsohn and McShane 2004). The Central Pacific and Indian Ocean have many cowries in common with the IWP, although some species are restricted to either one or the other ocean basins. Some species, such as *Monetaria caputserpentis* (Linnaeus, 1758) have teleplanic larvae and are widely distributed throughout most of the Indo-Pacific. Hawaii is more isolated and has fewer cowries than the Central Pacific, but some OTUs are restricted to the archipelago.

Discovery and documentation

Between 1758–1771 Linnaeus described 39 species (and the first few synonyms, in addition to one species now in the family Triviidae). His species include the most widespread
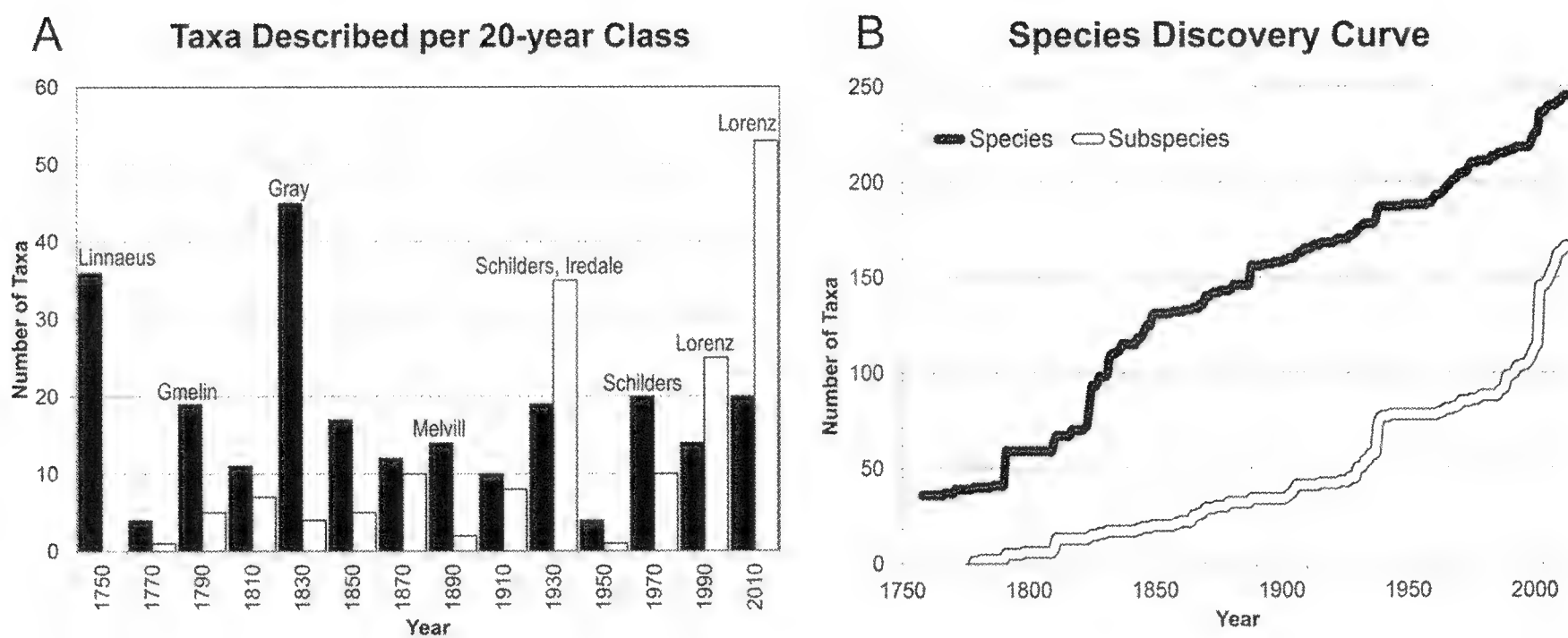
Figure 2. Species (black bars) and subspecies (white bars) descriptions in the Cypraeidae. **A,** Number of taxa described per 20-year class; some peaks are identified by the most prolific authors. **B,** Species discovery curve.

(*Monetaria caputserpentis*), the largest shell (*Macrocypraea cervus* (Linnaeus, 1771)), and some of the most common species in the family (*e.g.*, *Monetaria moneta* (Linnaeus, 1758)).

With the advent of oceanic navigation and world commerce, collection of shells and other natural history specimens turned into fashionable items in Europe. The drive to provide novelties eventually led to the most productive era, known as the "Golden Age of Discoveries," in the first half of the 19th Century, with John E. Gray being the most prolific author; the number of cowrie species more than tripled between 1800–1900. The rate of new species discovery remained steady since the mid-1800's to present (Fig. 2A–B), but the descriptions of new subspecies increased substantially since then, with an early peak in the 1930's thanks to the work of the Schilders and Tom Iredale. World War II took a toll also on new discoveries, but they resumed in the 1960's, again led by the Schilders.

The German couple Franz A. and Maria Schilder published over 400 papers during their nearly 50-year long career (Zeissler 1971), with close to 300 of them covering all aspects of the Cypraeacea, such as nomenclature and taxonomy (Schilder and Schilder 1938–1939, 1971, Schilder 1941), phylogeny (Schilder 1936), biogeography (Schilder 1965, 1969), fossils (Schilder 1932, 1935), shell morphometrics (Schilder and Schilder 1952, 1966), sexual dimorphism (Schilder and Schilder 1961, 1962), lumpers vs. splitters (*e.g.*, Schilder 1963), etc. However, they did not carry out field work themselves, and it has been said that only twice did they observe live cowries. That fact may have compromised their understanding of the importance of studying the living animal (Griffiths 1971).

Since the mid-1980's and continuing to date, another German, Felix Lorenz, has led the descriptions of new cowries. Lorenz is a biologist and shell dealer with access to large quantities of specimens, many self-collected; he has authored over 100 papers and several books (*e.g.*, Lorenz and Hubert 1993, 2000, Lorenz 2001, 2002) on cowries (and other mollusks). In the 21st Century, Lorenz has collaborated with molecular biologist Chris Meyer, by providing ample material for molecular studies (Meyer 2003, 2004), which uncovered a large cryptic diversity; many taxa were promptly described (*e.g.*, Lorenz 2002).

An analysis of the authors (and co-authors) of cowrie OTUs showed that the top seven authors have described about half of all OTUs, with Lorenz having described more OTUs than any other author, with 69 OTUs, followed F. A. Schilder (41), Linnaeus, Gray, Gmelin, M. Schilder, and Melvill (Fig. 3A). [The late Raybaudi Massilia, a shell dealer, named about 400 taxa, but most are invalid or synonyms (Groves and Weil 2002). The Schilders also named hundreds of taxa but most are infrasubspecific].

Additionally, although few cowries occur in the Mediterranean, the majority of taxa were described by Europeans (> 80%), and relatively few authors were from countries where cowries occur. Germany was the country that contributed the most OTUs (36%), thanks to the work of Gmelin, the Schilders, and Lorenz (Fig. 3B). About 93% of species were described by single authors, and
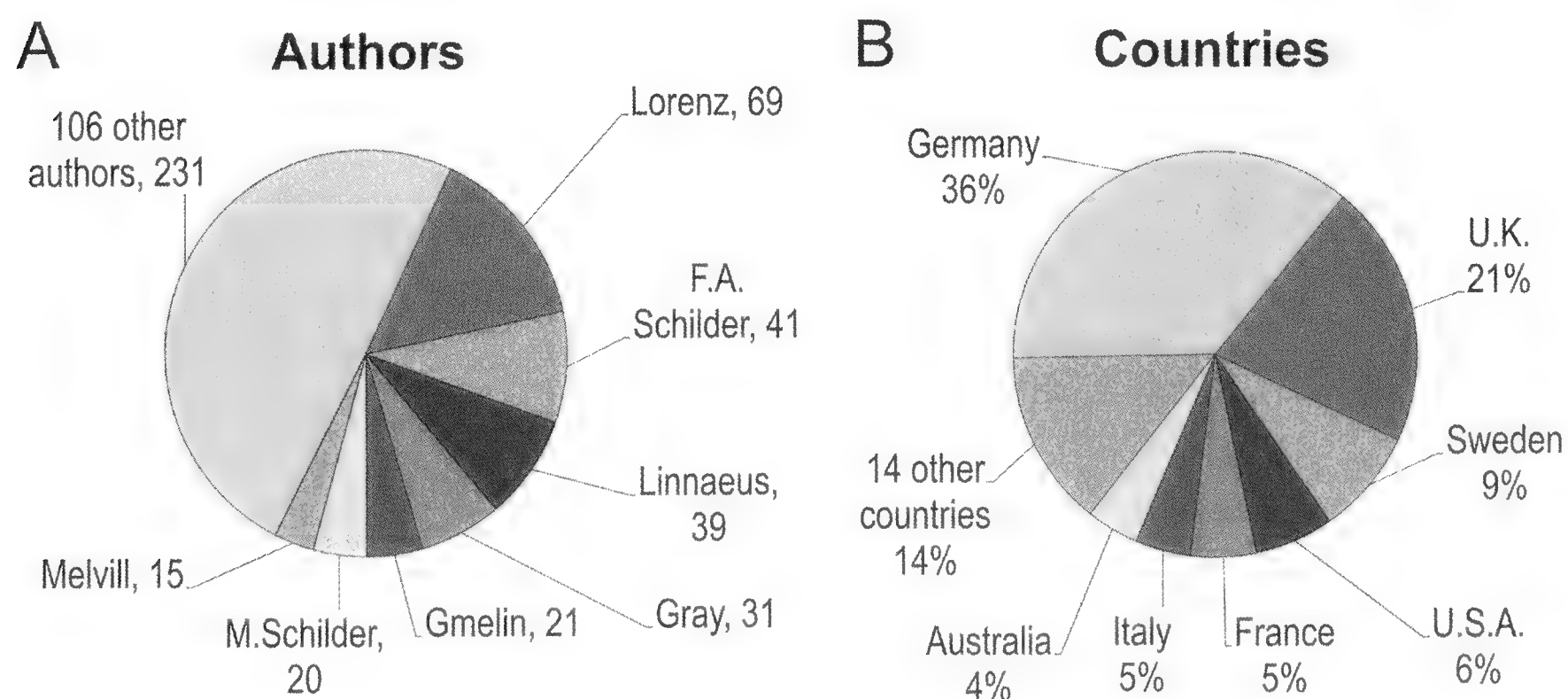
Figure 3. Authors and co-authors of OTUs in the Cypraeidae. **A,** The top seven most prolific authors and the number of OTUs they described that are considered valid. **B,** The top seven countries of origin of cowrie taxa authors; figures represent the percentage of taxa that were described by authors from each country.
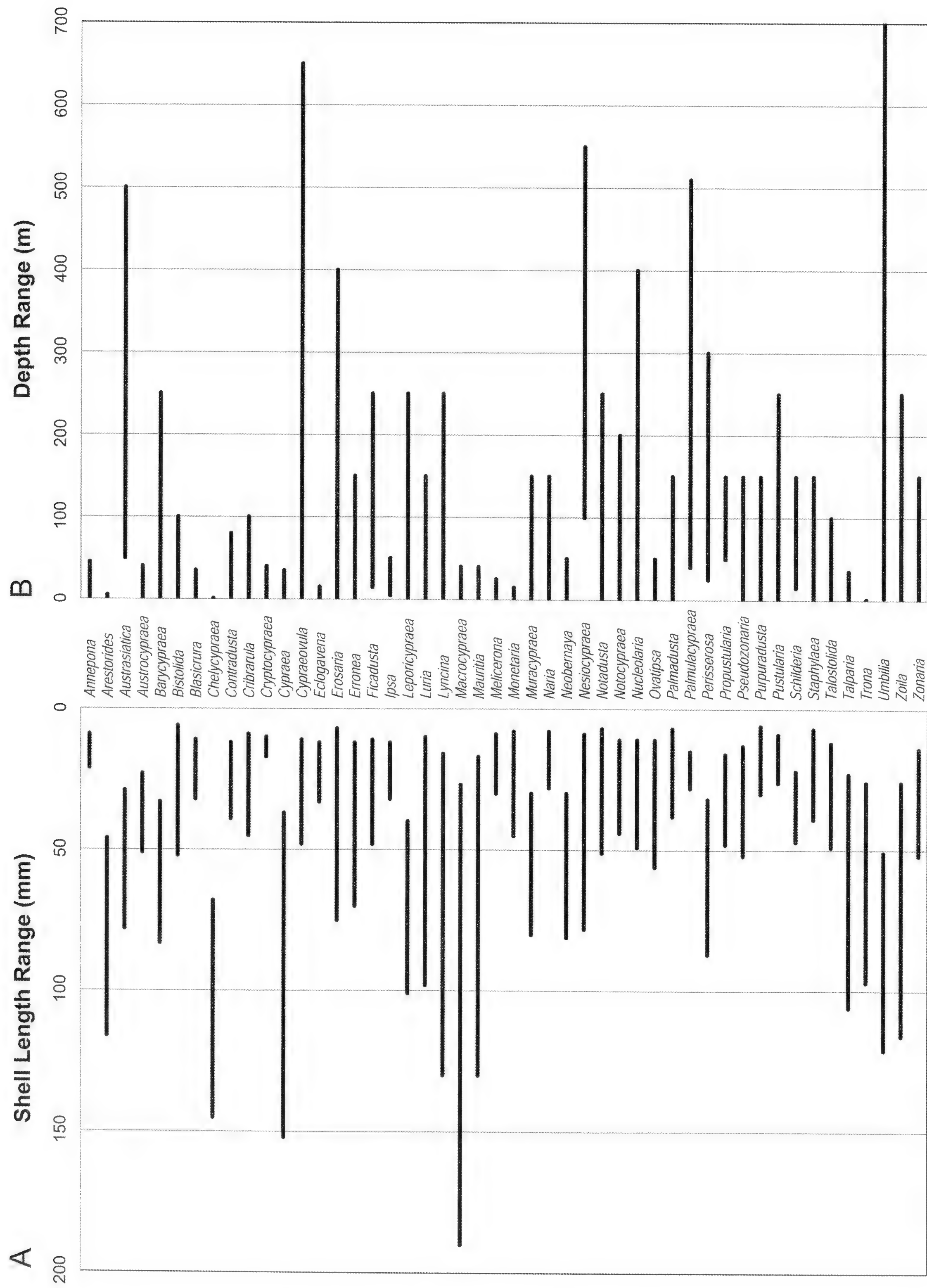
Figure 4. Ranges per genus. **A,** Shell length range (in mm) and, **B,** bathymetric range (in m).

7% by two authors, while 73% of subspecies were described by single authors, 25% by two authors, and 1% by three authors.

Shell length ranges were found for 317 OTUs, and from those, the modal shell length was about 25 mm long, with a range of 6–190 mm in the family. Five genera have OTUs with modal shell length of 15 mm or less; five genera have OTUs with modal shell length of about 70 mm. Ten genera have OTUs that can reach over 100 mm in shell length, while 22 genera have OTUs with a maximum length of 50 mm (Fig. 4A).

Bathymetric ranges were found for 282 OTUs; the modal depth was 5 m, with a range of 0–700 m in the family (Fig. 4B, Appendix 1). About 60% of the OTUs for which bathymetric range is known can be found in the intertidal zone or up to 2 m deep. Seven genera have species that reach 300 m or deeper (Fig. 4B). The deepest-living cowrie, *Umbilia capricornica* Lorenz, 1989, may reach a depth of 700 m (Lorenz and Hubert 2000).

DISCUSSION

Cowries are well known and their distributions have been well documented. Most cypraeid taxa have been described in non-peer review literature, based largely or solely on shell characters. Many new taxa are proposed with little scientific basis and upon closer scrutiny turn out to be synonyms of already known taxa, or represent anomalies in the normal range of intraspecific variation. Amateurs (defined as those not employed as practicing malacologists) have made important contributions to the cowrie studies such as describing new taxa, and providing material and information to malacologists. However, amateurs (and professionals) have also contributed a great deal of 'noise' to taxonomy with the introduction of unnecessary names. Taylor (1979) estimated that there were well over 1,000 names when less than 200 species were recognized, or over 5 synonyms per valid species in the Cypraeidae.

Besides the genuine desire to make scientific contributions, other motivations to describe new taxa include: 1) the vanity to have one's name attached to a taxon (either as describer or as a patronymic), and 2) economic gain. The latter is a well-known phenomenon: collectors pay a premium for any 'new' taxon (at least until it is disregarded as a synonym). These problems are neither new nor restricted to the cowries; many 'seashell' families (*sensu* Bouchet *et al.* 2011), butterflies, and other collectible organisms are also plagued with synonyms.

Many species have medium to large shells (Fig. 4A), and most cowries live in shallow waters (Fig. 4B), where they tend to be conspicuous reef-dwellers (albeit nocturnal), therefore, it is expected that most cowries have already been discovered. However, contrary to expectation, new species discoveries have continued on a steady pace since the 1850's to present. In contrast, subspecies descriptions were not common during the "Golden Age of Discoveries," but have become increasingly so from the 1930's to present. As a result, despite being one of the best-documented groups of mollusks, cowrie discovery curves (Fig. 2B) suggest that there may be still more cowries to be discovered. With the increased popular interest in shells, widespread scuba diving, expeditions in search of new discoveries (*e.g.*, Bouchet 2009, Bouchet *et al.* 2011), and the recent advent of molecular systematics, we are currently experiencing a "New Golden Age of Discoveries" (Fig. 2A), especially in non-'seashell' families (*e.g.*, Turridae, Pyramidellidae, Eulimidae, Galeommatidae, etc.), although description of new species is a challenge to taxonomists (Bouchet 2009). However, the cowrie discovery curves on Fig. 2B are likely to be inflated; once genera are reviewed, many recently-described taxa may turn out to be synonyms.

The new discoveries in cowries are expected to come from populations that: 1) hide in plain view as cryptic diversity and are likely to be discovered using molecular and/or detailed anatomical data; 2) live in deep water; 3) have limited distribution; and, 4) occur at the edges of distribution of known taxa. Included among the candidates with possible cryptic diversity are the seven genera with direct development, in particular *Cypraeovula* and *Zoila*, where large numbers of subspecies have been recognized (at least on a conchological basis) in populations with limited ranges. Further scrutiny, including anatomical and molecular data, is necessary to confirm the discoveries.

Meyer (2003, 2004) produced molecular phylogenies based on nearly comprehensive sampling of cowries from most known OTUs, only missing the more elusive OTUs. Overall, he found remarkable congruence with OTUs recognized by morphological characters and allopatry. Most of the so-called species complexes, genera or subgenera were found to be monophyletic with strong support. Meyer (2004) compared his results with the most recent checklist of the cowries at the time (Lorenz 2002) and about 93% of the species recognized by Lorenz were also Evolutionary Significant Units (ESU), and about 70% of the subspecies listed in Lorenz (2002) were recognized by molecular characters as ESU. In addition, 20 ESU showed cryptic diversity and had not been described by then (although most of them have been described by Lorenz since then).

The highest diversity of Cypraeidae occurs within the Coral Triangle (Fig. 1), the epicenter of marine biodiversity. The cowrie biodiversity gradient has a bull's eye pattern, with decreasing species richness as one moves away from the Coral Triangle, a pattern congruent with results from a study of over 10,000 marine species (about 80% of which reef fishes, but also mollusks, corals, crustaceans, seagrasses and mangroves) (Sanciangco *et al.* 2013). The Philippines and adjacent areas, with a complex patchwork of shallow and deep water habitats, are particularly rich in cowries. It is not surprising that most of the cowries (and seashells in general) in the shell collectors market come from the Philippines.

There are concerns about overexploitation of marine resources in the Philippines and elsewhere, especially because of the damaging effects of trawling (more for fish resources rather than shells). Most cowries occur within scuba and hookah diving range (Fig. 4B) and are collected manually by waders and divers, therefore, the damage to the habitat from the collection of shells is much lower than commercial fisheries or pollution. However, because cowries are valuable as specimen shells, especially the rare species, there is a great pressure where they occur in large numbers, and exploitation may be reaching unsustainable levels. More detailed studies are needed to assess the impact of commercial shell collecting on natural resources, and the conservation status of cowries.

Other topics that still remain largely unknown or which deserve further attention in cowries include: generic revisions and better characterization of genera on a morphological basis; ecology and study of live animals in the field to document behavior (*e.g.*, parental care of eggs) and life history; comparative anatomical studies; function of the mantle and its papillae; function of shell color and its secretion pathways (also the factors that influence "albinism", "melanism" and rostration); sexual dimorphism; cryptic species; hybridization; speciation and phylogeny, among others.

ACKNOWLEDGMENTS

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Appendix 1. Genera of the Cypraeidae with the type species, number of operational taxonomic units (OTUs), shell length range (mm), bathymetric range (m), and geographic distribution of each genus. Genera marked with an asterisk represent direct developers (Meyer 2003).

Genus	Type species	No. OTUs	Shell length range (mm)	Depth range (m)	Distribution
Annepona Iredale, 1935	*Cypraea annulata* Gray, 1828 (= *mariae* Schilder, 1927)	1	9–21	0–45	Pacific, East Africa
Arestorides Iredale, 1930	*Cypraea argus* Linnaeus, 1758	3	46–116	0–5	Indo-Pacific
Austrasiatica Lorenz, 1989	*Schilderia sakuraii* Habe, 1970	7	29–78	50–500	Pacific
* *Austrocypraea* Cossmann, 1903	*Cypraea contusa* McCoy, 1877 (Late Miocene)	1	23–51	0–40	NW to S Australia
* *Barycypraea* Schilder, 1927	*Cypraea (Aricia) caputviperae* Martin, 1899 (Late Miocene)	4	33–83	0–250	Arabian Sea to South Africa
Bistolida Cossmann, 1920	*Cypraea stolida* Linnaeus, 1758	25	6–52	0–100	Indo-Pacific
Blasicrura Iredale, 1930	*Cypraea pallidula rhinoceros* Souverbie, 1865	5	11–32	0–35	Indian Ocean to Central Pacific
Chelycypraea Schilder, 1927	*Cypraea testudinaria* Linnaeus, 1758	1	68–145	0–1	Indo-Pacific
Contradusta Meyer, 2003	*Cypraea walkeri* Sowerby I, 1832	2	12–39	0–80	Indian Ocean to Central Pacific
Cribrarula Strand, 1929	*Cypraea cribraria* Linnaeus, 1758	25	9–45	0–100	Indo-Pacific
Cryptocypraea Meyer, 2003	*Cypraea dillwyni* Schilder, 1922	1	10–17	0–40	Central Pacific
Cypraea Linnaeus, 1758	*Cypraea tigris* Linnaeus, 1758	3	37–152	0–35	Red Sea, Indo-Pacific
* *Cypraeovula* J. E. Gray, 1824	*Cypraea capensis* J. E. Gray, 1828	36	11–48	1–650	Southern Africa
Eclogavena Iredale, 1930	*Cypraea coxeni* Cox, 1873	6	12–33	0–15	Western Pacific
Erosaria Troschel, 1863	*Cypraea erosa* Linnaeus, 1758	34	7–75	0–400	Red Sea, Indo-Pacific
Erronea Troschel, 1863	*Cypraea errones* Linnaeus, 1758	28	12–70	0–150	Red Sea, Indo-Pacific
Ficadusta Habe and Kosuge, 1966	*Cypraea pulchella* Swainson, 1823	5	11–48	15–250	Indian Ocean to Western Pacific
Ipsa Jousseaume, 1884	*Cypraea childreni* Gray, 1825	2	12–32	5–50	Indo-Pacific
Leporicypraea Iredale, 1930	*Cypraea mappa* Linnaeus, 1758	7	40–101	0–250	Indo-Pacific
Luria Jousseaume, 1884	*Cypraea lurida* Linnaeus, 1758	9	10–98	0–150	Mediterranean, Indo-Pacific, Atlantic
Lyncina Troschel, 1861	*Cypraea lynx* Linnaeus, 1758	20	16–130	0–250	Red Sea, Indo-Pacific
Macrocypraea Schilder, 1930	*Cypraea exanthema* Linnaeus, 1767 (= *zebra* Linnaeus, 1758)	3	27–190	0–40	Eastern Pacific, Western Atlantic
Mauritia Troschel, 1863	*Cypraea mauritiana* Linnaeus, 1758	14	17–130	0–40	Indo-Pacific
Melicerona Iredale, 1930	*Cypraea listeri* Gray, 1824	3	9–30	0–25	Indian Ocean to Western Pacific
Monetaria Troschel, 1863	*Cypraea moneta* Linnaeus, 1758	6	8–45	0–15	Indo-Pacific
* *Muracypraea* Woodring, 1957	*Cypraea mus* Linnaeus, 1758	3	30–80	1–150	Venezuela and Colombia
Naria Gray, 1832	*Cypraea irrorata* (Gray, 1828)	2	7–24	1–150	Central Pacific
Neobernaya Schilder, 1927	*Cypraea spadicea* Swainson, 1823	1	30–81	0–50	California
Nesiocypraea Azuma and Kurohara, 1967	*Nesiocypraea midwayensis* Azuma and Kurohara, 1967	7	9–78	100–550	Eastern Africa to Western Pacific
Notadusta Schilder, 1935	*Cypraea victoriana* Schilder, 1935 (Late Miocene)	7	7–51	0–250	Eastern Africa to Central Pacific
* *Notocypraea* Schilder, 1927	*Cypraea piperita* Gray, 1824	7	11–44	0–200	Southern Australia
Nucleolaria Oyama, 1959	*Cypraea nucleus* Linnaeus, 1758	6	11–49	0–400	Indo-Pacific
Ovatipsa Iredale, 1931	*Cypraea chinensis* Gmelin, 1791	8	11–56	0–50	Indo-Pacific
Palmadusta Iredale, 1930	*Cypraea clandestina* Linnaeus, 1767	24	7–38	0–150	Red Sea, Indo-Pacific
Palmulacypraea Meyer, 2003	*Erronea (Gratiadusta) katsuae* Kuroda, 1960	5	15–28	40–510	West to Central Pacific
Perisserosa Iredale, 1930	*Cypraea guttata* Gmelin, 1791	2	32–87	25–300	Indian Ocean to West Pacific

Appendix 1. (Continued)

Genus	Type species	No. OTUs	Shell length range (mm)	Depth range (m)	Distribution
Propustularia Schilder, 1927	*Cypraea surinamensis* Perry, 1811	1	16–48	50–150	Florida to Brazil
Pseudozonaria Schilder, 1929	*Cypraea arabicula* Lamarck, 1810	5	13–52	0–150	Galapagos and Tropical Eastern Pacific
Purpuradusta Schilder, 1939	*Cypraea fimbriata* Gmelin, 1791	17	6–30	0–150	Indo-Pacific
Pustularia Swainson, 1840	*Cypraea cicercula* Linnaeus, 1758	15	9–26	0–250	Indo-Pacific
Schilderia Tomlin, 1930	*Cypraea utriculata* Lamarck, 1810 (Pliocene)	2	22–47	15–150	Mediterranean to Angola and Gulf of Biscay
Staphylaea Jousseaume, 1884	*Cypraea staphylaea* Linnaeus, 1758	8	7–39	0–150	Indo-Pacific
Talostolida Iredale, 1931	*Cypraea teres* Gmelin, 1791	8	12–49	0–100	Indo-Pacific and Eastern Pacific
Talparia Troschel, 1861	*Cypraea talpa* Linnaeus, 1758	2	23–106	0–35	Red Sea, Indo-Pacific
Trona Jousseaume, 1884	*Cypraea stercoraria* Linnaeus, 1758	1	26–97	0–1	West Africa
* *Umbilia* Jousseaume, 1884	*Cypraea umbilicata* Sowerby I, 1825 (= *hesitata* Iredale, 1916)	5	51–121	3–700	Southern Australia
* *Zoila* Jousseaume, 1884	*Cypraea scottii* Broderip, 1831 (= *friendii* Gray, 1831)	17	26–116	0–250	Southern Australia
Zonaria Jousseaume, 1884	*Cypraea zonaria* Gmelin, 1791	7	14–52	0–150	Mediterranean to West Africa
Average No. OTUs/genus		8.7 OTU/genus			
Range			6–190 mm	0–700 m	

Exploring the diversity of mesopsammic gastropods: How to collect, identify, and delimitate small and elusive sea slugs?*

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Abstract: Sediment-covered ocean floors constitute one of the largest and at the same time least explored habitats on Earth, still hiding an unknown level of species diversity. Coastal areas of this marine mesopsammic habitat harbor a variety of heterobranch snails and slugs. These gastropods long were puzzling due to their unclear phylogenetic positions, their aberrant morphologies and the lack of knowledge regarding their biology and diversity. Herein, we briefly review the advances of interstitial gastropod exploration, emphasizing that molecular approaches on formerly enigmatic mesopsammic groups like rhodopemorphs or acochlidians contributed to a drastic reconsideration of heterobranch systematics and evolution. We give an overview of the known diversity of mesopsammic heterobranchs and a list of type localities. In order to enhance surveys on the biodiversity of yet unexplored coasts, we then provide a suitable method to take samples of mesopsammic heterobranchs, and to extract and document slugs and snails from sands. A key based largely on externally-visible features allows for initial identification of already known taxa. Most mesopsammic gastropods show a "meiofaunal syndrome", *i.e.*, their morphology is constrained by the spatially-restricted interstitial environment, favoring rather uniform, worm-like body shapes and simple internal organization, which causes problems in conventional taxonomic approaches. Here, we present and discuss an integrative taxonomic workflow for delimiting potentially cryptic and elusive mesopsammic species, that also may be of use for other rare(ly) sampled invertebrates.

Key words: Acochlidia, field key, integrative taxonomy, Mollusca, Panpulmonata

THE MESOPSAMMON AND ITS INHABITANTS

Marine sediments and the interstices between sand grains, sometimes referred to as mesopsammon (Remane 1940), belong to the most ancient ecosystems of our planet (Rundell and Leander 2010). By the mid-19th and at the beginning of the 20th century, scientists discovered the water-filled interstitial space between the grains of coastal marine sands as a habitat for organisms (*e.g.*, Lovén 1844, Kowalevsky 1901a, 1901b, Giard 1904). Considerable progress has yet been achieved in different areas of meiofaunal research (*e.g.*, Remane 1952, Swedmark 1964, Ax 1969, Higgins and Thiel 1988a, Worsaae and Kristensen 2005, Giere 2009, Curini-Galletti *et al.* 2012, Worsaae *et al.* 2012). However, our knowledge of meiofaunal biodiversity, ecology and evolution is still limited and Rundell and Leander (2010) emphasized that the exploration of the meiofauna "remains among the most challenging, the most neglected and potentially the most enlightening frontiers of discovery in biology".

The interstitial milieu is characterized by extreme ecological conditions, such as faint light and limited amount of space, which restricts the body size and limits the interstitial fauna to minute, vermiform organisms suited to a lacunar environment (Swedmark 1964, 1968, Ax 1969, Higgins and Thiel 1988a). Currents and wave action transform the interstitial biotope by permanent restratification of the surface layer of the sand (Swedmark 1964). The continuous rearrangement of the particles contributes to a dynamic environment and makes the colonization by, *e.g.*, algae, difficult (Swedmark 1968). Furthermore, the living conditions in the intertidal zone or shallow water are complicated by diverse physical factors: the temperature varies with the time of day, seasons, and the rhythm of tides and, thus, fluctuates significantly in the surface layers of the sand layer; and, the salinity may increase by evaporation or decrease by rainfall or by the inflow of coastal freshwater (Giere *et al.* 1988). Organisms that successfully colonize the marine interstitial often develop special morphological and biological adaptations: body sizes are typically very small ranging from 0.5 mm to approx. 3 mm; flat

and broad or vermiform-elongated body shapes are commonly favored. The body wall is often reinforced by subepidermal spicules or cuticle for mechanical protection. Members of the interstitial fauna frequently have a strong contractibility and a high adhesive capability by different glandular systems to avoid being washed away (Swedmark 1964, 1968, Botosaneanu 1986, Higgins and Thiel 1988a). Consequently, the study of mesopsammic taxa is challenging—species are small, hard to collect, problematic to distinguish externally and difficult to describe by means of traditional techniques.

Different terms are associated with the fauna inhabiting marine sediments (see Higgins and Thiel 1988b, for summary). Most commonly a practical size definition is applied, characterizing all fauna which passes through a 1 mm mesh and is retained by 42 μm mesh as meiofauna (Higgins and Thiel 1988b). While this definition provides no relationship to a specific ecology and is also controversial due to deviations depending on e.g., anesthetized vs. living organisms, it is still of high practical value as directly related to extraction techniques in the field (Higgins and Thiel 1988b). Nearly all major metazoan taxa are represented in this size-defined marine meiofauna, e.g., Cnidaria, Platyhelminthes, Nemertea, Gnathostomulida, Gastrotricha, Rotifera, Annelida, Priapulida, Loricifera, Kinorhyncha, Acari, crustacean taxa, and Mollusca (e.g., Swedmark 1964, Botosaneanu 1986, Higgins and Thiel 1988a, Giere 2009, Rundell and Leander 2010). With the exception of Cephalopoda, all major molluscan clades include members that are at least temporarily meiofaunal (i.e., in early stages of their life cycle). Juveniles of molluscan taxa that inhabit soft sediments as adults (as sand-dwellers or epibenthic) are frequently encountered in sediment samples. Infaunal Scaphopoda and sediment-burrowing Caudofoveata are temporarily meiofaunal; few prochaetodermatid Caudofoveata retain meiofaunal sizes as adults (Morse and Scheltema 1988). The same applies for several Polyplacophora (pers. obs.; E. Schwabe pers. comm.), with currently only one species—Leptochiton intermedius (Salvini-Plawen, 1968)—being described as permanent meiofauna (Salvini-Plawen 1968). Among infaunal Bivalvia and Solenogastres, however, several representatives are known with permanently minute body sizes (e.g., bivalve Nuculidae and Mallettiidae (Poizat and Arnaud 1988) or neomeniomorph Meiomeniidae and Simrothiellidae (Morse and Scheltema 1988, García-Alvarez et al. 2000)). Among gastropod molluscs, we also frequently encounter temporarily meiofaunal forms in marine sediment samples. For example representatives of cephalaspidean Chelidonura A. Adams, 1850, nudibranchs Gymnodoris Stimpson, 1855, Aegires Lovén, 1844 and some chromodoridids, or some Runcinacea (Runcina Forbes [in Forbes and Hanley], 1851) were repeatedly observed in sand samples (pers. obs.). There are also several clades of shelled

gastropods (e.g., Pyramidellidae, Omalogyridae, Caecidae, Neritiliidae, Seguenziidae) with minute body sizes assigning them to permanent meiofauna. In most of these cases it is unknown, however, whether these snails lead an epibenthic or infaunal lifestyle.

The fauna inhabiting the interstices of sediment grains and which moves through its habitat with minimal disturbance (i.e., in contrast to organisms digging through the sand) is defined as 'interstitial' (Nicholls 1935) or 'mesopsammic' (Remane 1940) fauna. Usually this mesopsammic or interstitial fauna also falls in the size-defined category of meiofauna but not necessarily so when inhabiting very coarse sediments and shell gravel. Some 'typical' meiofaunal snails (sensu Arnaud et al. 1986) that are frequently extracted from bulk sediment samples may not be mesopsammic; instead they are surface dwellers that never venture deep into the sand (e.g., most Caecum Fleming, 1813 or Embletonia Alder and Hancock, 1851), or venture across the sand but live mostly on algae (Omalogyra Jeffreys, 1859, Runcinacea, large Rhodope Koelliker, 1847). In the present review we focus on meiofaunal and at least externally shell-less gastropods that show the characteristic adaptations of their body plan typical for interstitial taxa (see above) and which can, therefore, be considered as truly inhabiting the marine mesopsammon.

MESOPSAMMIC SLUGS PLACED IN A PHYLOGENY

Mesopsammic heterobranchs from different lineages (Fig. 1) often look quite similar to other meiofaunal "worms", having streamlined vermiform bodies and often lacking tentacles, body appendages or pigments, and even anatomically show similar organ reductions or special structures such as accessory ganglia or spicules. This phenomenon of particular morphoanatomical similarity caused by adaptation to a special environment was termed the "meiofaunal syndrome" by Brenzinger, Haszprunar et al. (2013). Multiple convergence in virtually all major organ systems causes problems not only for species identification and assignment of aberrant worm-like species to higher taxa, but especially for reconstructing their relationships in morphocladistic analyses (Schrödl and Neusser 2010).

Therefore, trials to recover the origin of mesopsammic heterobranch lineages using multi-locus sequence data appeared more promising and have, in hindsight, given invaluable contributions to heterobranch systematics. Vonnemann et al. (2005) first included three acochlidian species (representing both major subclades) into molecular analyses (18S and 28S rRNA), and failed to recover monophyletic opisthobranchs or pulmonates. Expanding the heterobranch taxon sampling and adding mitochondrial COI and 16S rRNA markers, Klussmann-Kolb et al. (2008) recovered a tree that
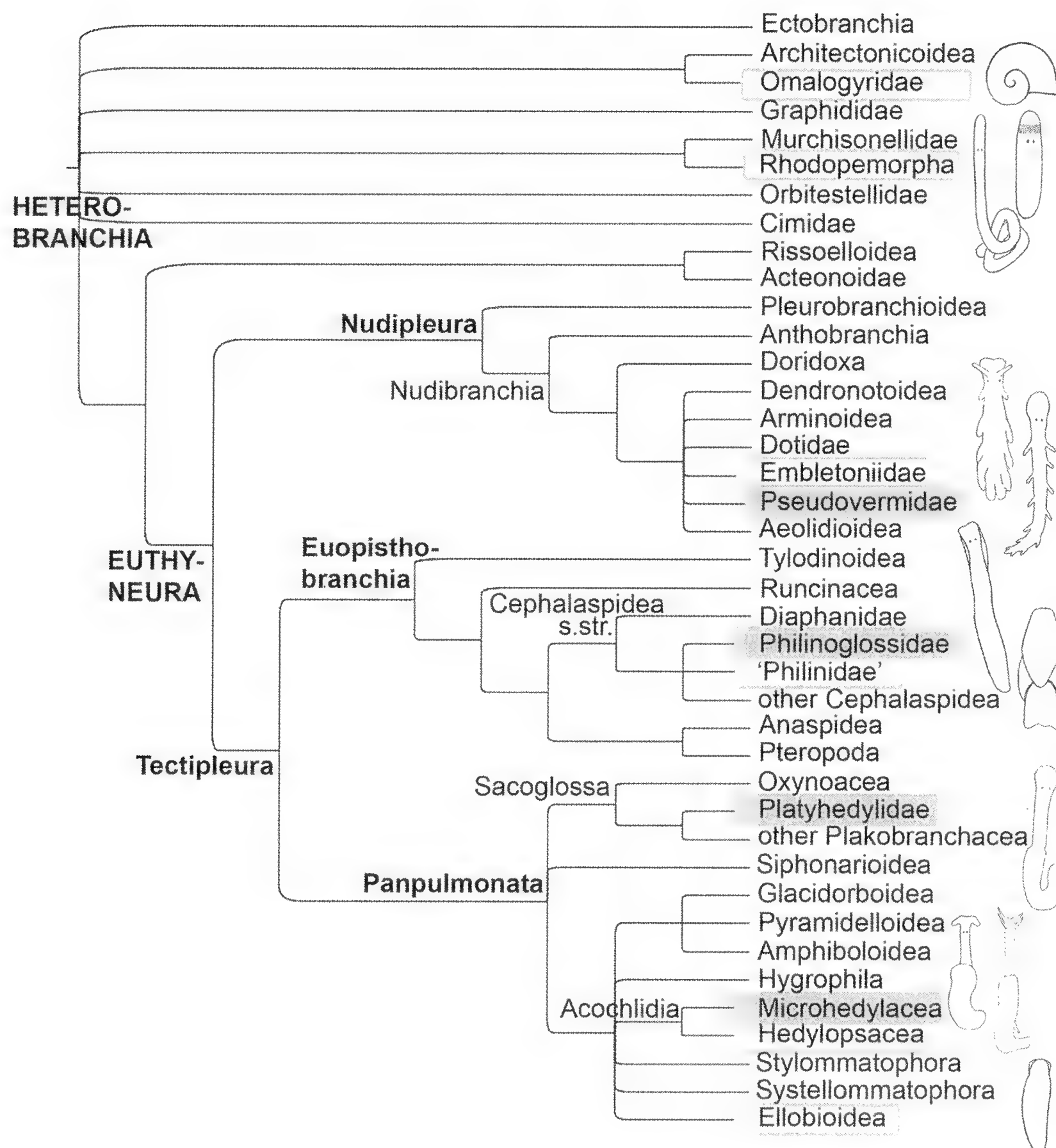
Figure 1. Living specimens of the major mesopsammic slug lineages. **A,** *Rhodope* sp. from Belize (Rhodopidae, Rhodopemorpha); **B,** *Helminthope* sp. from Papua (Rhodopidae, Rhodopemorpha); **C,** *Pseudovermis salamandrops* (Pseudovermidae, Aeolidoidea?); **D,** *Embletonia pulchra* (Embletoniidae, Aeolidoidea?); **E,** *Philine exigua* ('Philinidae', Cephalaspidea); **F,** *Philinoglossa marcusi* (Philinoglossidae, Cephalaspidea); **G,** *Platyhedyle denudata* (Platyhedylidae, Sacoglossa); **H,** *Pseudunela viatoris* (Pseudunelidae, Acochlidia); **I,** *Pontohedyle milaschewitchii* (Microhedylidae, Acochlidia). (Color shown in electronic version only).

1985, Dayrat and Tillier 2002), but the scientific community has long kept to a traditional concept of the two euthyneuran subtaxa, particularly because it conveniently bears a rough resemblance to the ecological division between sea slugs on the one hand and limnic and terrestrial slugs and snails on the other (Wägele *et al.* 2014). Based on multilocus data on a heterobranch taxon sampling including mesopsammic Philinidae, Philinoglossidae, Platyhedylidae, and six of seven acochlidian families, Jörger, Stöger *et al.* (2010) formally reclassified the Euthyneura. Major novelties were the exclusion of Acteonoidea, and the basal position of Nudipleura (with unsampled mesopsammic Pseudovermidae and Embletoniidae) sister to a clade with all other euthyneurans, termed Tectipleura (Schrödl *et al.* 2011). The latter divides into Euopisthobranchia, including Cephalaspidea *sensu stricto* with interstitial philinids and philinoglossids, and Panpulmonata, comprising sacoglossans (with mesopsammic *Platyhedyle* Salvini-Plawen, 1973) and Acochlidia related to siphonariids, pyramidellids, glacidorbids, amphibolids, and typical pulmonate groups (Fig. 2). Mesopsammic, extremely worm-like rhodopemorphs (Brenzinger, Wilson *et al.* 2011, Brenzinger, Haszprunar *et al.* 2013) surprisingly clustered with shelled and long-spired Murchisonellidae, which are basal heterobranchs (Brenzinger *et al.* 2014) in initial molecular analyses (Wilson *et al.* 2010). The new heterobranch tree is shown and discussed by Wägele *et al.* (2014).

From the perspective of interstitial fauna, heterobranchs invaded the mesopsammon at least eight times independently (Fig. 2), and in conclusion adapted to the environment convergently. Interestingly, and so far unique among interstitial gastropods, several acochlidian lineages reversed 'regressive evolution' characteristic for the mesopsammic fauna (Swedmark 1968; Westheide 1987) and

clearly contradicted Milne Edwards' (1848) classical division of the Euthyneura into Opisthobranchia and Pulmonata. Jörger, Stöger *et al.* (2010) reviewed potential morphological evidence for Pulmonata or Opisthobranchia, and could not find any. Retrospectively, Opisthobranchia or Pulmonata have never been well-supported monophyla (Haszprunar

Figure 2. Cladogram of Heterobranchia modified after Jörger *et al.* (2010b), combined with the consensus topology presented in Wägele *et al.* (2014), showing mesopsammic lineages in grey boxes (clades not entirely mesopsammic with grey outline only).

Hedylopsacea live epibenthic in marine, limnic, and (semi)terrestrial systems) (Schrödl and Neusser 2010; unpublished data). Pseudovermidae are entirely mesopsammic and currently comprise 16 valid species (Urgorri *et al.* 1991, Jörger *et al.* 2014). Seven mesopsammic species of cephalaspidean Philinoglossidae are currently valid and a single species is described from the mesopsammon for cephalaspidean Philinidae and Sacoglossa (Arnaud *et al.* 1986). In Rhodopemorpha it remains unclear whether the five species of *Rhodope* truly inhabit the mesopsammon or lead an epibenthic lifestyle; the monotypic *Helmithope* is considered a truly interstitial species based on its morphological adaptations (Brenzinger, Haszprunar *et al.* 2013). Five species of Smeagolidae are reported to inhabit cobble beaches (Tillier and Ponder 1992); given the large interstitial spaces of this habitat it probably differs considerably from the mesopsammic environment. Usually, mesopsammic slugs are rare and there are only few taxa in which higher local densities (> 100 individuals / 0.05 m^3) are reported (*e.g.*, some microhedylacean Acochlidia or philinoglossid Cephalaspidea (Poizat 1991)). The Acochlidia show worldwide distribution and present the only known lineage of mesopsammic sea slugs which also inhabit cold waters (*Asperspina murmanica* (Kudinskaya and Minichev, 1978) (see Neusser, Martynov *et al.* 2009)). All remaining lineages are restricted to tropical and temperate sands (Fig. 3); a compilation of type localities from all valid mesopsammic slugs is provided for future research in Appendix 1 (http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF). The comparably high diversity in European waters is likely a sampling artifact with a major effort of meiofaunal research focusing in Europe for decades (Coull and Giere 1988) and relatively little and isolated sampling effort in tropical zones (especially the Indian Ocean and large parts of the Indo-Pacific).

The sampling effort of our workgroup supported by a series of international collaborators have significantly raised the diversity in each of the lineages recovering a wealth of putative new species, which raises the number of species by up to ten fold (see Table 1). The presumably low reproductive output and dispersal abilities of meiofaunal slugs including Acochlidia suggests a high degree of endemism, which is supported by the detection of rather narrow ranges of distribution in many meiofaunal slugs and deep genetic divergence

reestablished an epibenthic lifestyle. Intertidal to supratidal Aitengidae adapted to semiterrestrial conditions (Neusser, Fukuda *et al.* 2011), the brackish-water adapted *Pseudunela espiritusanta* Neusser and Schrödl, 2009 lives under intertidal stones (Neusser and Schrödl 2009), and Acochlidiidae inhabit freshwater (*e.g.*, Wawra 1974, 1979, Brenzinger, Neusser *et al.* 2011).

DIVERSITY OF MESOPSAMMIC SLUGS

Compared to other meiofaunal taxa such as nematodes, polychaete annelids or copepods, all meiofaunal slug lineages are small taxonomic groups with few described species (Table 1). At current stage of research the Acochlidia are the largest clade of sea slugs inhabiting the mesopsammon with currently 26 valid species of Microhedylacea (an exclusively mesopsammic clade) and six Hedylopsacean species described from the interstices of sand grains (see Table 1, remaining

Table 1. Status of diversity of known meiofaunal slugs (Heterobranchia). ? No data available. *According to own unpublished data. **Hedylopsacea contain at least three secondarily non-meiofaunal lineages (Aitengidae, Acochlidiidae, and *Pseudunela espiritusanta*). *Rhodope* and *Embletonia* might be epibenthic and *Smeagol* inhabits cobble beaches. Anatomical characters do not necessarily fit the 'meiofaunal syndrome'.

Taxon	Described meiofaunal species/ Estimated total number including undescribed species*	References
RHODOPEMORPHA		
Rhodope Kölliker, 1847	6 / 15*	Haszprunar and Heß 2005; Wilson *et al.* 2010
Helminthope Salvini-Plawen, 1991	1 / 8*	Brenzinger, Haszprunar *et al.* 2013
NUDIBRANCHIA		
Pseudovermis Perejaslavtzeva, 1891	16 / 20*	Urgorri *et al.* 1991
Embletonia cf. *pulchra* Alder and Hancock, 1844)	1 / 10*	Miller and Willan 1992; Martynov 2007
CEPHALASPIDEA		
Philinoglossidae	7 / 15+*	Salvini-Plawen 1973; Brenzinger, Padula *et al.* 2013
Philine exigua Challis, 1969 and similar	1 / 5-10+*	Challis 1969
SACOGLOSSA		
Platyhedyle Salvini-Plawen 1973	1 / 4+*	Salvini-Plawen 1973; Rückert *et al.* 2008
ACOCHLIDIA		
Microhedylacea	26 / 40+*	Schrödl and Neusser 2010; Jörger and Schrödl 2013
Meiofaunal Hedylopsacea**	6 / 16+*	
OTINOIDEA		
Smeagol Climo, 1980	5 / 8?	Tillier and Ponder 1992; Fukuda and Ueshima 2010

in globally distributed lineages (Jörger *et al.* 2012). Moreover, the patchy occurrence typical for meiofaunal animals (*e.g.*, Poizat and Arnaud 1988, Andrade *et al.* 2011) can easily cause species to go undiscovered even in densely sampled areas (Curini-Galletti *et al.* 2012; pers. obs.). Considering the fact that the vast majority of marine sands, worldwide and in all varying depth ranges, are still virgin soil to meiofaunal research, the currently known diversity of mesopsammic slugs and probably also the reported unpublished findings in Table 1 still severely underrepresents true diversity. Overall, the contribution of meiofauna to marine biodiversity surveys has doubtlessly been underestimated, leaving this important ecosystem largely neglected in conservation approaches. We need fast, efficient and reliable means of species delineation in meiofaunal taxa to address this taxonomic impediment—means that are capable of dealing with the putatively high degree of cryptic speciation likely to be the rule for meiofauna (Jörger *et al.* 2012). Accounting for the still exploratory stage of meiofaunal research, we aim to contribute in the following

to exploring worldwide mesopsammic sea slugs by providing guidelines on how to extract specimens from sediments and a key for initial determination of mesopsammic sea slugs into major taxa at least, updating Arnaud *et al.* (1986). Moreover, we discuss the pitfalls of species delimitation in meiofaunal slugs, evaluate the pros and cons of various species delimitation methods, and propose an integrative work flow that especially addresses the needs of mesopsammic and other taxa with only few samples available.

INSTRUCTIONS TO STUDY MESOPSAMMIC SLUGS IN THE FIELD

Searching and extracting mesopsammic sea slugs

In the course of our studies we conducted sampling trips to different biogeographic zones for initial exploration of the interstitial malacofauna, and experienced very heterogeneous
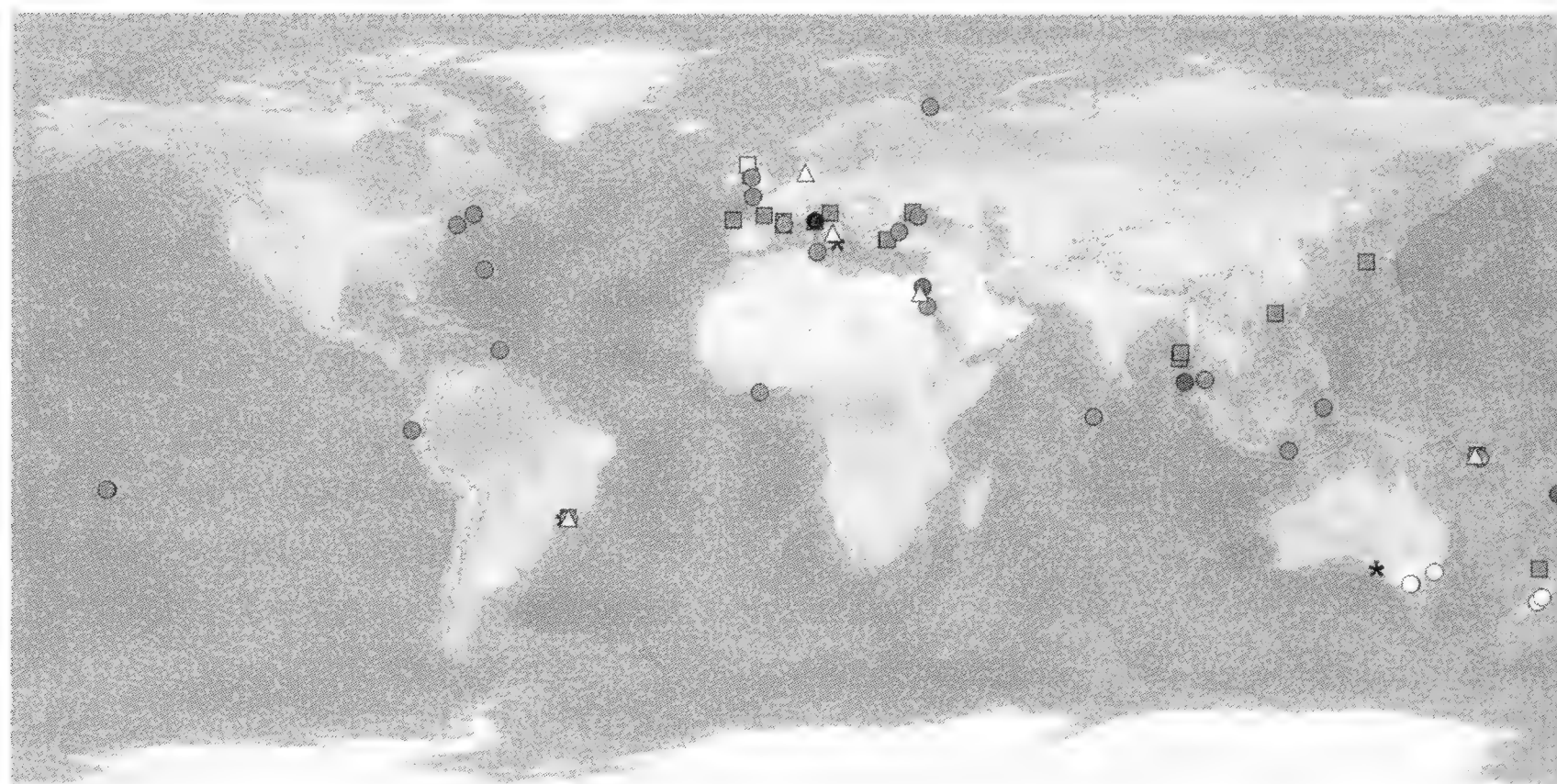
Figure 3. Overview of the type localities of the different mesopsammic microslugs (for details see also Table 1). 'Lower heterobranch' Rhodopemorpha marked with black star. Nudipleuran taxa marked with squares (dark grey: Pseudovermidae, light grey: Embletoniidae). Euopisthobranch Cephalaspidea shown in triangles (light grey: Philinoglossidae, dark grey: Philinidae). Panpulmonate clades marked as dots (dark grey: hedylopsacean Acochlidia, light grey: microhedylacean Acochlidia, white: Sacoglossa, black: Smeagolidae).

regional density of specimens and species diversity. In general, subtropical or tropical coasts seem much more species-rich than cold waters (compare in Fig. 3), and not all depths or sediment types host the same or an equally rich gastropod meiofauna. For example, in our experience lava sands are generally poorer with respect to meiofaunal gastropod diversity then coral sands. Usually, coarse oxygenated subtidal substrates with steady water currents appear privileged in species diversity relative to intertidal, wave-exposed fine sandy beaches or fine sediments with much organic content (Poizat and Arnaud 1988; pers. obs.). We now know of specialized acochlidians from all continents but Antarctica, from many islands regardless their distances to continental coasts, and even from high energy beaches or brackish water-influenced estuaries. Other mesopsammic groups such as cephalaspideans also occur in finer, detritus-rich sediments, often sporadically or seasonally (Poizat 1984). Freshwater-influenced sediments may host acochlidians and sacoglossans, and, so far, a single known acochlidian species (*Tantulum elegans* Rankin, 1979 from the Carribbean) even occurs in sediments of a swampy mountain spring (however, our own recollecting attempts at the type locality in 2009 failed).

In summary, almost all our sampling trips resulted in finding a variety of mesopsammic sea slugs and snails, often including lineages new to science (see above). To encourage and support sampling of mesopsammic slugs, we here provide a suitable and detailed step-by-step procedure to extract mesopsammic molluscs from sand samples (modified from Pfannkuche and Thiel (1988) and updated from Schrödl (2006) and Neusser (2011)) and list suggestions based on our

experience on how to anesthetize and fix encountered specimens for various purposes (Appendix 2) (http://www.bioone. org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF).

Documenting mesopsammic slugs in the field

During specimen collection, researchers should consider that samples have to be treated and selected wisely, and prepared differently according to later, various uses. In absence of an external shell, the taxonomy of meiofaunal slugs or snails with reduced internal shell was mainly based on external morphology of living or preserved specimens, presence and type of calcareous spicules and radula characteristics (Kowalevsky 1901b, Arnaud *et al.* 1986, Wawra 1987). Snails and slugs may distort or retract during fixation if not relaxed very carefully, pigments may fade especially in ethanol, and calcareous spicules will disappear quickly in any calcium carbonate undersaturated or acidic fixative (Poizat and Arnaud 1988). Live documentation of ephemeral morphological features and of behavior and movement, thus, is a unique opportunity to study phenotypes and essential to preliminarily identify new findings and to correlate them with existing taxonomy. Of special interest are—next to lucky occasional observations on, *e.g.*, feeding or reproduction—the behavior during disturbance (*e.g.*, ability to retract) and the behavior in motion (*e.g.*, the ability to adhere to the substrate). Important taxonomic characters in meiofaunal slugs are the general body shape, shape and relative size of head appendages (*i.e.*, oral tentacles, rhinophores), body appendages (*i.e.*, cerata in nudibranch *Pseudovermis* and *Embletonia*), and relative length and width of the foot; all of which should preferably be documented on living material to avoid fixation artifacts. Observations of internal anatomy (*e.g.*, (different types of) spicules or pigments (including eyes)) are most informative using carefully squeezed, anesthetized specimens under transmission light microscopy, preferably with differential interference contrast (DIC). Whenever possible, photographs and high-resolution videos of living animals should be used to later identify and describe internal taxonomic characters (*e.g.*, minute, thin-walled structures like the heart can easily collapse during preparation for histology and detection might be easier on living material than on histological sections). Radulae of meiofaunal species are resistant to fixation and preservation, but very small and structural details may not be adequately revealed using light microscopy requiring further analyses via scanning
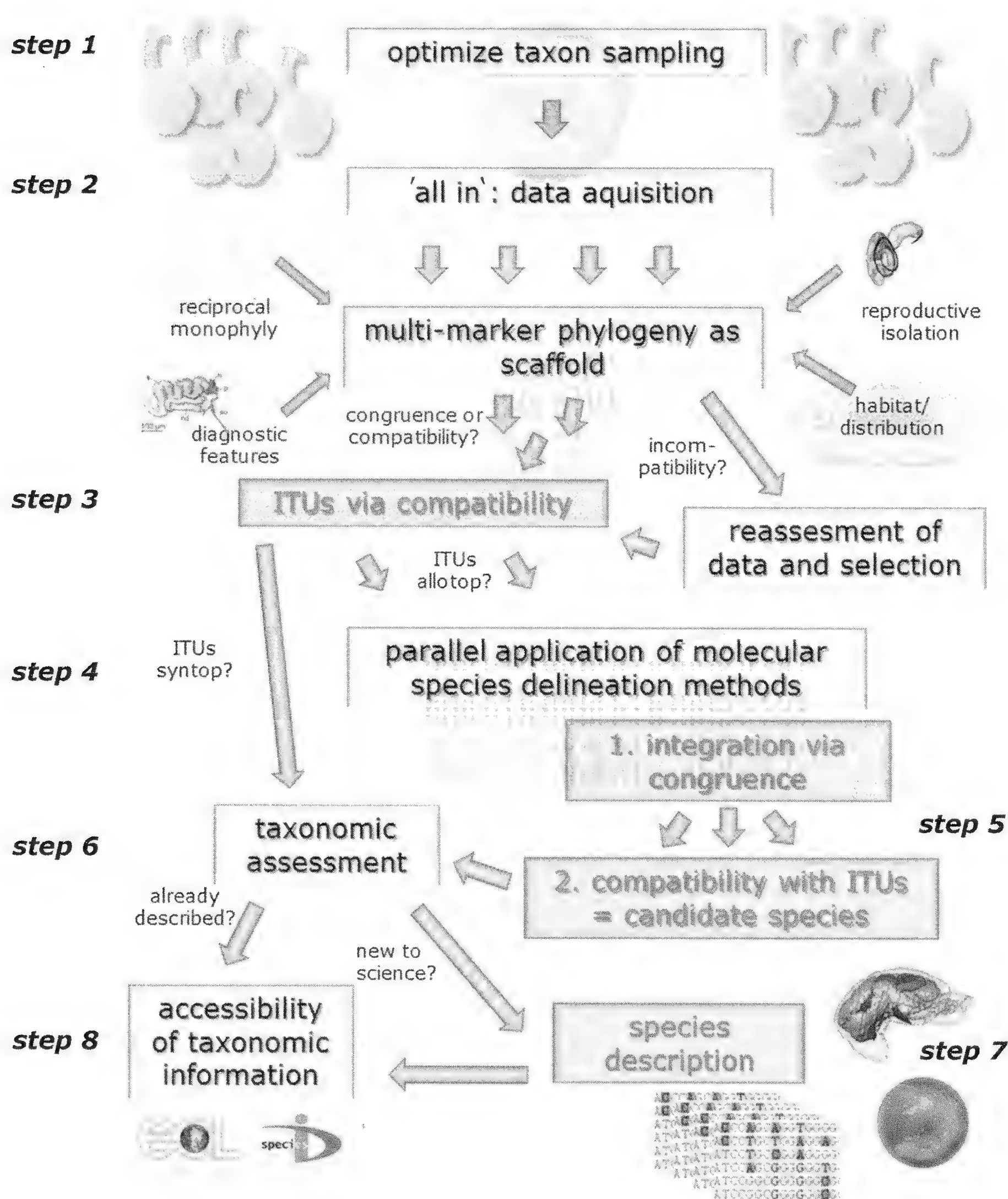
Figure 4. Flowchart on the proposed workflow on species delineation in elusive taxa, modified after the approach by Jörger *et. al.* (2012). (Color shown in electronic version only).

mesopsammic lineages and their descendants and close relatives (*e.g.*, Neusser *et al.* 2006, 2007, Jörger *et al.* 2008, Rückert *et al.* 2008, Jörger *et al.* 2009, Neusser, Heß *et al.* 2009, Neusser, Martynov *et al.* 2009, Brenzinger, Wilson *et al.* 2011, Kohnert *et al.* 2011, Brenzinger, Haszprunar *et al.* 2013, Brenzinger, Padula *et al.* 2013, Kohnert *et al.* 2013, Jörger *et al.* 2014) and to reconstruct the evolution of phenotypes and biology from molecular approaches. This contributes to a better knowledge of the diversity in the mesopsammon and an evaluation of the diagnostic characters for identification of these taxa. As reviewed above, sampling efforts in the past years have discovered a series of species potentially new to science, which partially differ unequivocally from all described mesopsammic slug lineages by characters of the external morphology (see *e.g.*, Fig. 1A). On the other hand, the new material from the mesopsammon largely comprises cryptic lineages (especially within morphologically static Microhedylacea), which could only be revealed as novel evolutionary entities through the use of integrative approaches employing 3D-microanatomical descriptions and molecular data (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012, Jörger and Schrödl 2013), see discussion on species delimitation below. Nevertheless, many sea slug lineages inhabiting the interstitial—known and still unknown ones—may be tentatively identifiable to higher taxonomic categories such as family or genus-level in the field using the key presented herein (Appendix 3, http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF). For observing external features a dissecting microscope is necessary and internal features such as presence and structure of shell, spicules or radulae requires a transmission light microscope.

electron microscopy (SEM). Since preparation of minute radulae is challenging (Geiger *et al.* 2007), prior adequate documentation via light microscopy is indispensable. Rather than preparing whole mounted specimens for soft part anatomy, we recommend recovering the documented individuals and fix them for molecular studies or advanced morphological techniques (see Appendix 1, http://www.bioone.org/doi/suppl/10.4003/006.032.0205/suppl_file/Jorger_2014_suppl.PDF) for different fixatives successfully applied).

A key to identify mesopsammic slugs

There are ongoing efforts to investigate in detail the microanatomical and morphological diversity of described

SPECIES DELIMITATION IN MEIOFAUNAL GASTROPODS – AND OTHER RARE AND ELUSIVE TAXA

Morphological species delineation

Traditionally, the taxonomy of gastropods relies on external morphological characters; even in 2006, approximately 80% of new gastropod species descriptions were based solely on shell characters combining the advantages of unproblematic preservation in natural history collections and potential for *post mortem* identification (Bouchet and Strong 2010). However, several studies with largely molecular scope have demonstrated the potentially high intraspecific variability of both shell morphology (*e.g.*, Hauswald *et al.* 2008, Bouchet and Strong 2010, Puillandre, Modica *et al.* 2012) and external characters in general, such as color variation in slugs (Nitz *et al.* 2009). When representatives of meiofaunal slug lineages were first discovered, their aberrant external morphology was in many cases sufficient for species delimitation (*e.g.*, Kowalevsky 1901a, 1901b). This changed with every new discovery of other closely related meiofaunal gastropods, making further characteristics of radulae and spicules obligatory for species delineation within clades (Salvini-Plawen 1973, Arnaud *et al.* 1986, Wawra 1987). In restricted geographic areas, a combination of these characters might still be sufficient to diagnose mesopsammic slugs (Eder *et al.* 2011), but on a broader scale these characters become insufficient (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012). External features in mesopsammic slugs are heavily constrained by the requirements of the spatially restricted habitat and provide little variation. Other features—such as the presence of externally visible eyes—show high intraspecific plasticity (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012).

For most gastropods the radula morphology is of major importance for taxonomy, in meiofaunal slugs diagnostic characters are, however, often limited to minute details (*e.g.*, the number of lateral denticles on the rhachidian tooth in Pseudovermidae (Urgorri *et al.* 1991)). Therefore, light-microscopic investigation might be insufficient to reliably analyze the minute radulae of meiofaunal gastropods and reinvestigation by SEM is needed for reliable comparative analyses. Due to the partially minor interspecific variation, intraspecific and intraindividual variation requires special attention and in some cases radula characteristics may be insufficient to diagnose species (*e.g.*, Jörger *et al.* 2014).

Anatomical data included in species descriptions were traditionally based on morphological data from squeezed whole mounts (Kirsteuer 1973), whole mount or crush preparation of the radula (*e.g.*, Doe 1974) and/or the examination of histological sections of up to 10 µm thickness, which were frequently paraffin-based and distorted (*e.g.*, Odhner 1937, Marcus 1953, Challis 1968, Challis 1970, Morse 1976), and, therefore, not always reliable considering modern standards. Traditional manual reconstruction techniques from semithin histological sections (Sommerfeldt and Schrödl 2005) are time-consuming and challenging. We currently consider a 3D-based microanatomical approach most powerful: because modern 3D reconstructions based on µCT and synchrotron microtomography data currently do not allow for detailed microanatomical investigation in micromolluscs (Kunze 2013), we favor an approach using AMIRA software to reconstruct 3D models of all major organ systems based on serial semithin histological sections of 1–1.5 µm thickness (method after Ruthensteiner (2008); for methodological discussion see also DaCosta *et al.* (2007)).

Redescriptions of all major meiofaunal slug lineages based on advanced 3D-microanatomy in conjunction with ultrastructural data from, *e.g.*, sperm have compiled microanatomical characters across all organ systems, and these characters have proven reliable for taxonomic purposes (Neusser *et al.* 2006, Neusser and Schrödl 2007, Jörger *et al.* 2008, Neusser, Martynov *et al.* 2009, Jörger, Kristof *et al.* 2010, Martin *et al.* 2010, Brenzinger, Wilson *et al.* 2011, Eder *et al.* 2011, Kohnert *et al.* 2011, Brenzinger, Padula *et al.* 2013). These studies demonstrated the high quality of modern morphological approaches, which provide reliable, highly detailed diagnostic characters for taxonomic and systematic studies.

But even high-end morphological study reached its limits when confronted with the extraordinary degree of convergent adaptation that gastropods are notorious for (Ponder and Lindberg 1997, Dayrat and Tillier 2002, Wägele *et al.* 2014); and this adaptation is carried to an extreme in taxa that inhabit environments such as the mesopsammon, which selects for certain morphological and anatomical adaptations. Moreover, 3D-microanatomical approaches can be very time-consuming and taxonomists are faced with a trade-off between detailed accounts on a small number of specimens and estimations of the intraspecific vs. interspecific variability of characters. Therefore, in cases with ambiguous morphological data—which is the rule rather than the exception in mesopsammic slugs—only integrative approaches that combine evidence from morphology and molecules represent a viable method for tackling their diversity (Neusser, Jörger *et al.* 2011, Jörger *et al.* 2012). Given the putative high degree of cryptic speciation in meiofaunal taxa with supposedly low dispersal abilities (*e.g.*, Westheide and Schmidt 2003, Casu *et al.* 2009, Fontaneto *et al.* 2009, Leasi and Todaro 2009, Andrade *et al.* 2011, Jörger *et al.* 2012, Tulchinsky *et al.* 2012), it currently seems most efficient to reverse the traditional taxonomic workflows and initiate species delineation in meiofauna with barcoding and molecular species delineation approaches and integrate morphoanatomical and other data (rather than initiating with morphoanatomical lines of evidence and integrate molecular data).

Molecular species identification and delineation

DNA barcoding and molecular species delineation have been broadly advocated as fast and efficient means for dealing with the taxonomic impediment in times of biodiversity crisis (Blaxter *et al.* 2004, Blaxter *et al.* 2005, Hebert and Gregory 2005, Markmann and Tautz 2005, Hajibabaei *et al.* 2007). DNA-barcoding in its similarity-based form, which uses genetic distances, is a tool of species (re-)identification and not species discovery (DeSalle *et al.* 2005, DeSalle 2006). Lacking a predictive component, DNA-barcoding fails when no identical sequences are deposited in public databases (like Barcode of Life Data System http://www.boldsystems.org/ or GenBank http://www.ncbi.nlm.nih.gov/genbank/). Ongoing efforts of the workgroup include depositing barcodes of all valid mesopsammic slugs to public databases to allow for identification via barcodes (current coverage approx. 60%, partially still unpublished). However, as discussed above the vast majority of marine meiofauna have yet to be explored (Curini-Galletti *et al.* 2012), not to mention identified and sequenced, identical matches of newly collected material with deposited sequences will be the exception for meiofaunal taxa for decades to come (Jörger *et al.* 2012). Meiofaunal biodiversity assessments, therefore, will not be focused in typical DNA barcoding identification approaches, but require advanced methods of molecular species discovery.

Most of the numerous emerging programs and algorithms that have recently become available for molecular species delineation either rely on the comparison of genetic distances or use phylogenetic trees to estimate support under different model assumptions. To cluster sequences based on genetic distances, programs either use fixed or relative thresholds between intraspecific and interspecific variation (*e.g.,* Hebert *et al.* 2004, Jones *et al.* 2011, Ratnasingham and Hebert 2013) or aim to detect breaks in patterns of distance distribution, *i.e.,* a 'barcoding gap' (Meyer and Paulay 2005, Puillandre, Lambert *et al.* 2012). Distance-based approaches are usually based on mitochondrial cytochrome *c* oxidase subunit I (COI); this standard barcoding marker presents unique species-specific diagnostics in approximately 95% of all tested species. Moreover, the interspecific differences clearly exceed the intraspecific variability in most of these cases (Hebert, Cywinska *et al.* 2003, Hebert, Ratnasigham *et al.* 2003, Ratnasingham and Hebert 2013). Despite high success rates in praxis, the use of thresholds as proxy of species delimitation has been criticized for their arbitrariness; and criticism has been underscored by empirical studies demonstrating the disappearance or absence of a 'barcoding gap' (*i.e.,* intraspecific exceeds interspecific variability) with increased sample size (Moritz and Cicero 2004, Meyer and Paulay 2005, Wiemers and Fiedler 2007, Astrin *et al.* 2012). Model-based approaches such as the General-Mixed-Yule-Coalescent (GMYC) model infer evolutionary entities by evaluating likelihood values under speciation vs. population genetic processes on phylogenetic trees (Pons *et al.* 2006, Monaghan *et al.* 2009). But the accuracy of this and other model-based approaches also relies on sampling coverage (Lohse 2009). A dense sampling coverage is usually utopic when it comes to elusive taxa, whose sampling records frequently include a high degree of singletons. The rarity of taxa in undersampled datasets hampers reliable estimation of intraspecific vs. interspecific variation, and constitutes the primary obstacle of successful molecular species delineation in elusive taxa. Currently, a Bayesian approach evaluating for differences among gene trees is potentially best capable of dealing with singletons, provided that data from several independent markers are combined (Yang and Rannala 2010, Zhang *et al.* 2011). In the absence of a 'gold standard' for evaluating the performance of different species delineation approaches, and in view of high degrees of rarity in putatively undersampled datasets, an integrative approach of species delineation is needed for elusive taxa, one which allows for thorough cross-validation between approaches.

Workflow of integrative species delineation

Herein, we present a workflow capable of dealing with the above-mentioned problems, which are likely symptomatic for most meiofaunal taxa and other little explored and rare taxa such as, *e.g.,* many deep-sea clades, or many invertebrates in general. Due to the putative high degree of cryptic speciation and intraspecific variability on morphological character sets, the workflow is founded on molecular data. Faced with incomplete datasets and rarity, however, it is designed to make best use of the taxonomic information scattered across different lines of evidence. The proposed workflow (see Fig. 4) is based on the approach described in Jörger *et al.* (2012).

Step 1: Optimize taxon sampling and character sampling

In concordance with previous species delineation workflows (*e.g.,* Puillandre, Modica *et al.* 2012), this approach emphasizes the importance of dense taxon sampling to ensure reliable species delineation and to avoid overestimating interspecific variability (Hebert *et al.* 2004). In elusive taxa, taxonomists frequently lack knowledge on biology, dispersal abilities and geographic distribution, which requires an even stronger emphasis on targeted taxon sampling with regard to both geography and phylogenetic relationships. This includes collecting and analyzing several individuals from populations covering the potential geographic range of a taxon. Hence this workflow requires an *a priori* survey of the described species of a lineage; and whenever possible, material of valid species derived from type material or specimens re-collected at type localities should be included.

Under the unified species concept, species are defined as independently evolving metapopulation lineages, with the former secondary species criteria of competing concepts serving as equal operational criteria, *i.e.*, lines of evidence (de Queiroz 2005b, 2007). As central operational criteria serve intrinsic reproductive isolation, monophyly, exclusive coalescence, diagnosibility and deficits of genetic intermediates; the reliability of proposed species hypotheses increases with the number of supporting lines of evidence (de Queiroz 2005b, 2007). Entities discovered in molecular species delineation approaches should, therefore, be supported by a minimum of one line of evidence. Consequently, the workflow requires the grouping of all characters sets which were evaluated and selected by the taxonomist as contributions to one of these operational criteria (*e.g.*, molecular data, morphological and anatomical characters with special emphasis on reproductive features, geographic distribution, behavioral data, ecological niches). Therein, the workflow strongly encourages to initially give equal priority to all putatively useful character sets.

Step 2: 'All-in' – plotting of data onto a molecular phylogeny

Studies in species delineation of elusive taxa such as mesopsammic slugs should aim to gather as much putatively relevant information from as many different sources as possible (*i.e.*, morphoanatomical, ecological, molecular, and so on). Despite all efforts to compile 'complete' data matrices, in reality some populations will provide exhaustive information, while others will be represented by singletons only. Furthermore, amplification success may vary among samples, species and individuals, as bulk fixation of sediment samples usually degrades DNA. And finally, immature specimens can prevent the exploration of reproductive features and body retraction or damages often occur. In typical broad-scale barcoding approaches for biodiversity assessments, amplification problems of the COI-barcoding marker can result in incomplete, ambiguous sequences that are unable to pass quality filters on automated pipelines on Barcode of Life Data systems (BOLD) (Ratnasingham and Hebert 2013), thus, causing a potentially 'correct' lineage to be irretrievably lost for further assessments. To ensure the inclusion of all available lineages in this species delineation approach in spite of missing data, the scattered information on individual lines of evidence must be compensated by the amount of (self-contained) lines of evidence. Therefore, and to account for problems of incompatibility between species and gene trees (caused mainly by incomplete lineage sorting, pseudogenes, or introgression (Bensasson *et al.* 2001, Funk and Omland 2003, Song *et al.* 2008), this approach is based on 'multi-barcoding', including independently evolving markers (ideally from mitochondria and nucleus) (Jörger *et al.* 2012). As this approach is based on phylogenetic theory via the criterion of monophyly, single gene trees are calculated from each individual marker. The risk of producing artificial

topologies is minimized by using Bayesian and/or Maximum Likelihood algorithms, rather than rapid distance based methods. Each gene should be checked for reticulate evolution (*e.g.*, using Dendroscope 3 (Huson and Scornavacca 2012)). Then, a concatenated all-marker phylogeny serves as scaffold for plotting other sources of data (focusing on those that putatively serve as operational criteria).

There are two major advantages of this unfortunately time-consuming initial step: 1) The unvalued objective plotting of the available characters of all terminals (or at least populations, if there is no doubt on conspecificity) in the dataset without biased pre-selection is based either on initial (single-gene) molecular data or on taxonomic intuition relying on, *e.g.*, morphological criteria. A prefiltering of available data into morphotypes (Riedel, Sagata, Suhardjono *et al.* 2013) leaves potential cryptic species undiscovered and is, therefore, not advisable for meiofaunal taxa. 2) The potential for quality checks and cross-validation between different lines of evidence. This critical reassessment of the primary data can help to reveal problematic molecular markers or potential homoplasies on morphological characters.

Step 3: 'Wild cards' and selection of integrative taxonomic units (ITUs) via compatibility.

Based on the plotted data, integrative taxonomic units (ITUs) are defined: Integrative taxonomy is commonly considered best practice (Dayrat 2005, Will *et al.* 2005, Valdecasas *et al.* 2008, Padial and de la Riva 2010, Padial *et al.* 2010, Astrin *et al.* 2012, Riedel, Sagata, Suhardjono *et al.* 2013), but approaches differ considerably in how exactly they integrate their data, with far-reaching consequences for the resulting species hypothesis. Typical large-scale barcoding workflows cluster COI sequences into operational taxonomic units (OTUs) based on genetic distances via different algorithms, and they encourage the addition of accessory data from other molecular markers or *e.g.*, morphology (Jones *et al.* 2011, Puillandre, Modica *et al.* 2012, Ratnasingham and Hebert 2013). When more data is added, the species diagnosis becomes integrative, whereas species delineation, which has led to the discovery of the OTU, remains based on a single line of evidence and will not be questioned or critically revised by additional data.

When truly integrating data into the process of species delineation, there is debate on the degree of congruence that different characters need to provide (Padial *et al.* 2010). Integrating via congruence requires a minimum of two selected lines of evidence to support the proposed species hypothesis, while in 'integration via cumulation' approaches the divergence of any character can justify the designation of species (Padial *et al.* 2010). The former approach promotes taxonomic stability, but implies the risk of underestimating species numbers. Integrative taxonomy via cumulation, on the other hand, tends to overestimate species, but is thereby likely best

suited to discover recent lineages (Padial *et al.* 2010). Ideally, ITUs can be selected across the integrative scaffold established in this workflow via congruence across all lines of evidence, *i.e.*, reciprocal monophyly supported by morphological features and geographic and habitat boundaries. For small datasets, concordance between operational criteria can be evaluated by eye, but especially in larger datasets, the use of statistical methods for testing of support values as developed by Cardoso *et al.* (2009) is advisable. The herein presented workflow suggests a less stringent application of congruence, promoting integration via compatibility, *i.e.*, allowing for entities supported by some and uncontradicted by other lines of evidence (see the 'minimum consensus' approach in Jörger *et al.* (2012)). This accommodates the fact that the process of speciation does not necessarily implement changes on all different levels of characters (Padial and de la Riva 2010, Padial *et al.* 2010) and that methods of detection have different sensitivities, but the approach remains conservative in relying solely on uncontradicted support for a particular species hypothesis.

The effects of speciation patterns may, however, result in incongruent datasets (Padial and de la Riva 2010, Padial *et al.* 2010). Integrative taxonomy, thus, must not be misunderstood as simply adding more and more data, rather it urges cautious selection of the appropriate character set for the species under investigation (Valdecasas *et al.* 2008). Faced with incongruence, the debate on which character set is best suited to species delineation cannot be solved universally, but has to be decided in each individual case with regard to the specifics of each taxon.

At this point the proposed workflow offers a potential short-cut to species assignment: reciprocally monophyletic clades occurring in syntopy, *i.e.*, in the same biotope, can be considered species under the unifying species concept, combining the operational criteria of intrinsic reproductive isolation with reciprocal monophyly (de Queiroz 2005a, 2007). The evaluation of syntopy in meiofauna is problematic, however, due to the largely unknown ecological interactions and potentially small-scale microhabitats.

Although less conservative than integrative taxonomy via (strict) congruence, the compatibility approach will nevertheless tend to lump species. Because it relies on the criterion of reciprocal monophyly, this initial step is likely not suited for detecting recently evolved lineages (Knowles and Carstens 2007, Sauer and Hausdorf 2012). It, therefore, needs to be refined in Step 5 in order to uncover any potential lumping of species.

Step 5: Parallel application of available methods of molecular species delineation

As discussed above, the accuracy of all available algorithms of molecular species delineation suffers from undersampled datasets and the inclusion of singletons (Jörger *et al.* 2012) and

also tends to oversplit datasets in empirical studies (*e.g.*, Sauer and Hausdorf 2012). In the absence of a 'gold-standard' for comparing the performance of each analysis on the respective dataset, our workflow suggests an unbiased parallel application of available molecular species delineation methods across all markers. Special emphasis should be given to model-based approaches such as GMYC (Pons *et al.* 2006, Monaghan *et al.* 2009)—provided that minimum requirements, *e.g.*, on sampling density, are fulfilled—and algorithms capable of dealing with rarity, such as Birky's (2013) simple coalescence theory based approach. The inference of genetic connectivity via haplotype networks applying statistical parsimony (Clement *et al.* 2000) is merely an indirect method of estimating species boundaries (Pons *et al.* 2006); nevertheless, it visualizes the genetic structure in the dataset, which is valuable for cross-validating molecular entities revealed by other approaches. Even though the performance of distance-based approaches is conceptually disputed and practically hindered in lineages that putatively suffer from incomplete sampling (Meyer and Paulay 2005, Hickerson *et al.* 2006, Meier *et al.* 2006, Wiemers and Fiedler 2007, Meier *et al.* 2008), the parallel application of Refined Single Linkage analysis (RESL) (Ratnasingham and Hebert 2013) or the Automatic Barcode Gap Discovery (ABGD) (Puillandre, Lambert *et al.* 2012) contributes to the empirical evaluation of the efficiency of standard barcoding approaches on elusive taxa. All the above methods are applied to single genes, and single gene histories may differ and, thus, results need to be compared and their significance evaluated. Using concatenated markers is not appropriate since information from single loci may be dominant and mask potential conflict. A powerful approach based on multilocus markers is the Bayesian Species delineation (Yang and Rannala 2010, Zhang *et al.* 2011); the more independent markers are available for combination, the better it can deal with rarity of samples.

Step 6: Congruence in molecular species delineation and compatibility of ITUs to determine candidate species

To exploit the potential of molecular species delineation methods for revealing prior lumping of species, integration via compatibility is inapplicable in this step, as it would directly transfer over-splitting of each individual method to the identification of the candidate species. The workflow aims for a cross-validation between the different approaches achieved by integrating the results via congruence. Only molecular entities supported by all molecular species delineation approaches are then further integrated via compatibility to the formerly identified ITUs, in order to lead to the final determination of the candidate species.

Step 7: Assessment of the taxonomic history

With regard to ecological studies or biodiversity assessments, an advantage of rare or elusive taxa compared to common or

hyperdiverse clades is the fact that the former usually lack an exhaustive history of available descriptions and putative synonyms, with the majority of lineages being still undescribed. To avoid the creation of synonyms, it is crucial to clarify whether the discovered candidate species already bears a valid name, *i.e.*, a thorough literature review on all potential names available. If several names are available, the oldest name has priority according to nomenclatural rules. If old descriptions do not permit unambiguous assignment, we recommend using the oldest name that can be reliably assigned to the newly delimitated species (Ornelas-Gatdula *et al.* 2012). Alternatives would be to resurrect unused or dubious older names, which is a rather arbitrary procedure, or to deliberately establish and name a "new" species despite the existence of already available names, thus, creating a junior synonym.

Step 8: Species description

Independently evolving lineages discovered as candidate species, but which cannot be assigned to valid species, should be described to receive formal recognition. Molecular species delineation approaches frequently terminate their efforts with the discovery of independently evolving lineages (*e.g.*, Fontaneto *et al.* 2009, Monaghan *et al.* 2009, Astrin *et al.* 2012). The BIN system (Barcode Index Numbering on BOLD) even propagates the use of OTUs as an alternative taxonomic reference system (Ratnasingham and Hebert 2013), drawing on initial proposals on DNA taxonomy (Tautz *et al.* 2003). The amount of deposited sequences, which are unidentified at the species level and bear other unique identifiers, has grown tremendously in the course of the barcoding endeavor over the past few years (see http://iphylo.blogspot.de/2011/04/dark-taxa-genbank-in-post-taxonomic.html). Clustering sequences into OTUs may be sufficient for further applications such as biodiversity assessments, while unidentified sequences can still contribute valuable information in the absence of species assignment. The name of a species is entirely extrinsic, it could, thus, be replaced by any alternative identifier such as a BIN. However, the use of OTUs not merely as a source of taxonomic characters, but also as a taxonomic reference system, can be problematic when it comes to establishing novel unique identifiers, as these identifiers create parallel taxonomies flagged with new acronyms and classification systems in competition with traditional taxonomy (Jörger and Schrödl 2013). The Linnaean name anchors the species to its taxonomic history and all available biological and morphological data (Polaszek *et al.* 2008, Patterson *et al.* 2010). Moreover, the genus name includes a hypothesis on phylogenetic relationships. A species name can be linked to life science identifiers via ZooBank (http://zoobank.org/), capable of uniquely linking content on this species through different computational platforms (Polaszek *et al.* 2008). In order to reduce and not enhance impediments in taxonomy by parallel yet inconsistent identifiers, discovered lineages should be connected

to the taxonomic history of a clade by providing formal descriptions (Jörger and Schrödl 2013). Depending on the chosen operational criteria of species delimitation, this set of characters will form the basis for the diagnoses of species. Jörger and Schrödl (2013) illustrated how molecular diagnostic characters can be extracted via character-based barcoding approaches (Sarkar *et al.* 2008, Bergmann *et al.* 2009) and used for taxonomic description. Future efforts should aim to automate the extraction of diagnostic molecular characters to facilitate and accelerate species description, as has already been achieved in 'turbo-taxonomic' approaches for morphological data (Butcher *et al.* 2012, Riedel, Sagata, Suhardjono *et al.* 2013, Riedel, Sagata, Surbakti *et al.* 2013). However, the fair option to diagnose species entirely based on molecular characters, morphological data should nevertheless be included in the species description (Jörger and Schrödl 2013).

Step 9: Ensure accessibility of all data

Digital technologies provide powerful methods for making taxonomic data more accessible to the research communities via, *e.g.*, virtual access to museum collections, digitalized biodiversity libraries, online registration systems for zoological names and infrastructure for biogeographic assessments (*e.g.*, Wheeler 2008, Padial and de la Riva 2010, Padial *et al.* 2010). Next to the obligatory voucher deposition in museum collections (including vouchers of extracted DNA) and of genetic sequences in public databases, data from species descriptions can now be deposited in online platforms (*e.g.*, the Encyclopedia of Life http://eol.org/). This increases the accessibility of taxonomic knowledge and allows for dynamic expansion of species diagnoses through future studies (Riedel, Sagata, Suhardjono *et al.* 2013), ideal for gradually augmenting knowledge on enigmatic taxa.

CONCLUSIONS AND OUTLOOK

The marine mesopsammon is one of the largest, yet least explored habitats on Earth, and the taxonomic deficit is correspondingly high. To date, the mesopsammic fauna has been largely neglected in conservation approaches, despite their doubtless important role, *e.g.*, in the marine food web. The comparably low reproductive output and the poor dispersal abilities of mesopsammic slugs indicate small ranges of distribution and high degrees of endemism. The threat to their diversity by habitat destruction is consequently high. By providing practical sampling instructions on how to explore the mesopsammic malacofauna in the field, we aim to encourage the inclusion of this fauna into biodiversity assessments. A boost in sampling efforts world-wide—but with special emphasis to the numerous unsampled tropical regions—is urgently needed to get reliable estimations on the diversity of

these enigmatic taxa still hidden in global sands. Currently, the micromorphological diversity of known microslugs is comparatively investigated and the inclusion of minute mesopsammic sea slugs into multi-locus analyses on Heterobranchia has demonstrated how theses enigmas can help to understand the phylogenetic relationships and evolution of larger clades. We now need to fill in the gaps in existing taxon samplings with remaining elusive taxa (*e.g.*, Pseudovermidae) in order to supplement the complex picture of heterobranch evolution step by step and to understand the evolutionary pathways which led into the mesopsammon.

We emphasize that next to the initial exploration in the field, there is also a theoretical debate needed on how to efficiently and reliably delineate meiofaunal species—a task which usually presents a struggle with rarity and uniformity. Despite all valuable advances in accelerating the rates of taxonomic descriptions to face the 'taxonomic impediment' in times of biodiversity crisis, here we promote a form of 'deep taxonomy' in cases where the evolutionary history of species requires a thorough integrative workflow. This will ensure that these small clades do not slip through automated 'turbo-taxonomy' pipelines.

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A new critical estimate of named species-level diversity of the recent Mollusca*

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Abstract: Modern estimates of species-level diversity in the recent Mollusca range from 34,000 to 120,000 described species, with total diversity including undescribed species often cited as 200,000. Most estimates are unverifiable, not being based on reproducible methods. Ultimately the best way to gauge diversity is explicit enumeration: actual listing of known species. Comprehensive lists of species are valued as a basis for systematic revisions and for comparing diversity across taxa, but it is less appreciated that they also provide a means for statistical sampling of biodiversity databases. I assessed the completeness of molluscan species listings in the World Register of Marine Species (WoRMS) by comparing it to a standardized inventory of the species represented in the Malacology collection of the Academy of Natural Sciences of Philadelphia (ANSP). Random samples of names were scored for presence or absence in WoRMS, with standard errors calculated from the binomial distribution. The WoRMS database has about 1,200 duplicate or extraneous listings for mollusk species and is missing about 1,300 (3%). Overall marine molluscan diversity is estimated at 43,600 ± 900 species, where 900 is a 95% confidence interval. The validity of this confidence interval depends on the WoRMS database and the ANSP collection not having correlated weaknesses. Lack of relatively complete species databases prevents similar assessments for terrestrial and freshwater mollusks, but, using less rigorous methods, I estimate that there are 70,000 to 76,000 described species of recent Mollusca.

The low end of this estimate, 70,000 species, is similar to the number of recent chordate species, 69,000, so it is possible that the Mollusca are not the second most diverse phylum of animals in terms of recognized recent species. Naming rates for chordate are currently higher than for mollusk, 750 versus 600 species per year, although the Mollusca are regarded as having higher undescribed diversity. The Mollusca have long been considered the most species-rich marine phylum, but the estimate of 43,600 is substantially below the 56,000 species of Arthropoda listed in WoRMS. Globally, the ratio of marine gastropod to marine bivalve species in WoRMS is 4:1, which is higher than in any regional fauna, suggesting that gastropods have smaller geographic ranges on average than bivalves. The main remaining gaps in WoRMS are among opisthobranchs (8% missing) and Indo-Pacific marine mollusks (6%). The most diverse molluscan genus in WoRMS is *Turbonilla*, with more than 1,000 species listed as accepted.

Key words: biodiversity, gastropod, bivalve, databases, random sampling

There have been many prior estimates of species-level diversity of recent mollusks, as summarized by Boss (1971) and Chapman (2011). Modern estimates of the number of named species range from 34,000 (Boss 1971) to 120,000 (Ponder *et al.* 2002) and of total diversity, including undescribed species, from 47,000 (Boss 1971) to the often cited 200,000 (Wells 1995, Groombridge and Jenkins 2002, Chapman 2011). My own estimates for described species have been 72,000 (sum of diversity by classes in Rosenberg 1992, p. 11–12) and 85,000 (cited by Chapman 2011, p. 34, as pers. comm.).

Most estimates do not state the methods used and are not verifiable, being essentially expert opinions, or summaries thereof. For example, Mayr (1969) listed molluscan diversity as 107,250 species without stating the source of his estimate. At the same time he listed avian diversity as 8,600 species, stating (p. 12): "In birds 99 percent of all living species have surely been described". Yet the number of bird species now recognized exceeds 10,500 (International Ornithologists' Union <http://www.worldbirdnames.org/>), an increase of 22%. Some of this gain, however, represents the elevation of subspecies and synonyms to full species rather than discovery of new species.

The uncertainty in estimating diversity in a well-known taxon such as Aves illustrates the need for considerable caution in estimating molluscan diversity. Figure 1 shows that

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the species discovery curve for marine mollusks has no sign of an asymptote. It is dangerous to extrapolate from such a curve, because it can change trajectory without any more species being described. Currently recognized taxa might be synonymized, and synonyms might be recognized as representing valid taxa. These changes would not be graphed in the year the classification changed, but in the year the taxon was named. Figure 1 does not show the number of species that were recognized in 1950 (for example); it shows the number of species currently recognized that had been named by 1950 (leaving aside the problem of renamed homonyms). Since extrapolating an asymptote in this situation is untenable, Figure 1 is best interpreted as showing that fewer than half of the species of marine mollusks have yet been discovered, since a point of inflection has not been reached on the curve.

Rather than trying to estimate the number of mollusk species that might ultimately be discovered, I focus here on determining how many have already been described. The best method for doing this is explicit enumeration, as advocated by Sykes (1901), that is, actual listing of known species. In general, estimates of molluscan diversity on the lower end take this approach, counting named species across taxa and faunas (Boss 1971). Figure 1 shows the results of explicit enumeration of species in the World Register of Marine Species (WoRMS, http://www.marinespecies.org/).

Since 2008, WoRMS has emerged as a global standard for marine molluscan taxonomy. Its main molluscan editors initially were Philippe Bouchet, Serge Gofas and me, with Bruce Marshall and Rüdiger Bieler joining in 2013 and 2014 respectively. More than 30 other editors have contributed to various families and superfamilies of Mollusca. Records created or last edited by taxonomic editors appear online as "verified" (with a green check mark) and records added or last edited by data management personnel (perhaps imported from another database or from uncritical checklists) appear as "unverified" (with a red question mark). The online database shows the editing history of each record, with the name of the person responsible for each change and a date-time stamp.

Comprehensive lists such as WoRMS are valued for providing the nomenclatural basis for systematic revisions and for comparing diversity across taxa, but it has been less appreciated that they also provide a means for statistical sampling of biodiversity databases. Although WoRMS is approaching completeness for valid species, it is less complete for synonyms. Also, it has some duplicate listings, and some that are out of scope (freshwater, terrestrial or fossil, but not flagged as such). I present methods here for understanding the scale of these problems in a statistical framework, along with methods for estimating how many named marine taxa are missing, and for estimating the diversity of terrestrial mollusk species in the absence of a global list of species.

The computerized inventory of the mollusk collection at ANSP has been a useful tool in this process. Since the beginning of 2011, the current placement in the collection of about 320,000 lots has been recorded in the department's collection database, including almost all of the gastropods. The inventory standardizes names across the database (for spellings and generic placements), allowing random sampling based on name. A taxonomic dictionary that links to the genus field gives the higher classification for each name. This dictionary also gives the habitat for each genus: as one or more of "marine", "brackish", "freshwater" and "terrestrial", which allows random sampling by those descriptors. For gastropods, the ANSP inventory is largely independent of the WoRMS database, as most work was completed before the WoRMS database was regarded as complete enough to guide curation, however, there are some areas of overlap. Triphoridae in WoRMS was compiled with reference to the ANSP collection and Patellogastropods at ANSP were curated against WoRMS.

The results for species diversity are similar to those I presented at the symposium in 2012, but have been entirely recalculated since October 2013 when I
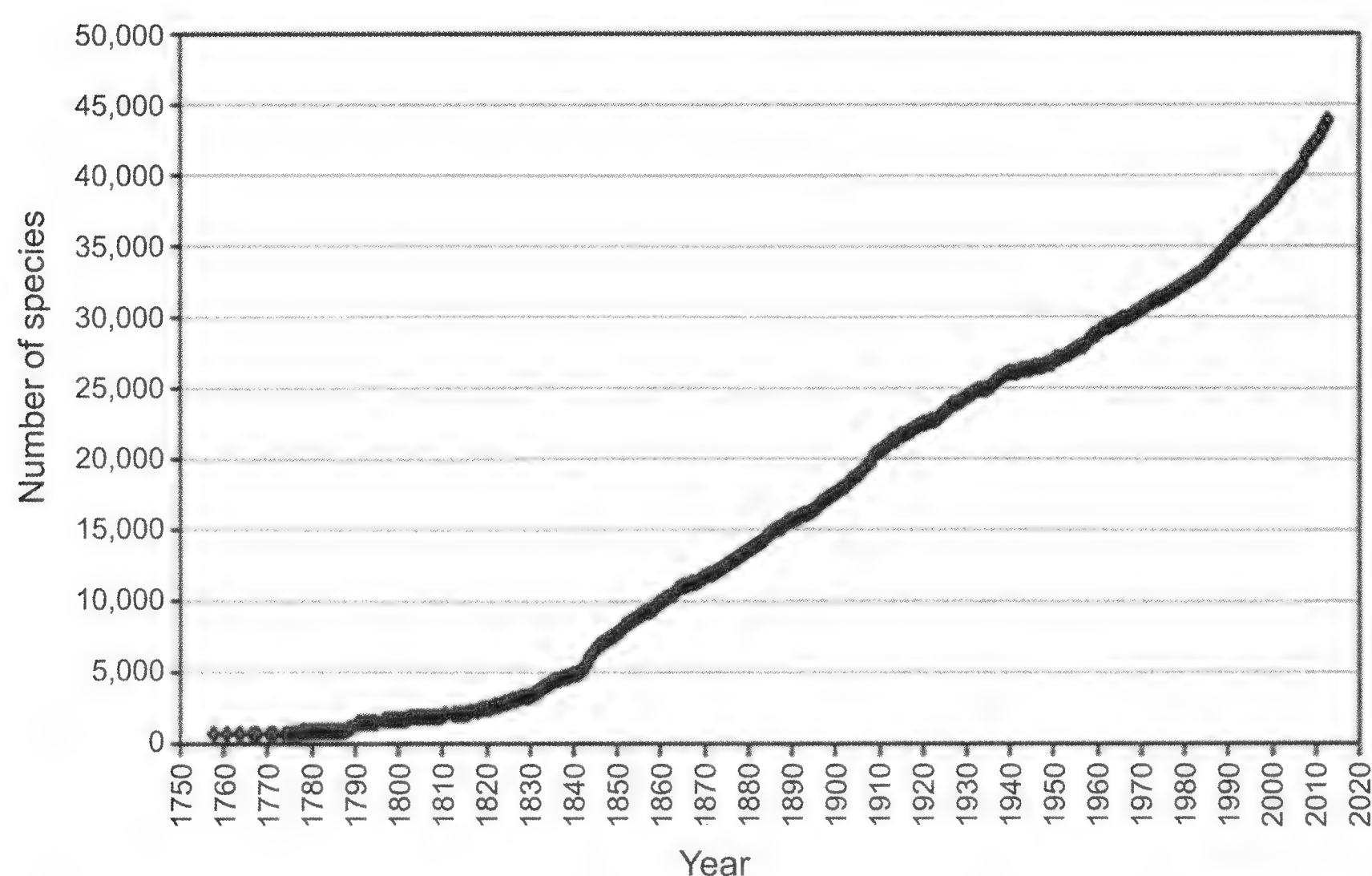
Figure 1. Species discovery curve showing species currently treated as valid in WoRMS cumulatively by year of description.

completed a major push to add data to WoRMS, focusing particularly on the Pyramidellidae, which had been underrepresented. Most of the molluscan classes had relatively little growth in that period (0–1.4%), but the Gastropoda grew by more than 10%, not including species named since June 2012. I had estimated then that WoRMS might be missing as much as 16% of gastropods, so closing that gap has been important for producing a more reliable estimate of marine molluscan diversity.

MATERIALS AND METHODS

To estimate error levels in WoRMS, I took "verified" and "unverified" names (see Introduction) of accepted species of Bivalvia and Gastropoda at random and queried based on the specific epithet to determine how many were duplicates, synonyms, or out of scope (*e.g.*, fossil only, freshwater only). "Duplicates" were defined as the same epithet applied to the same taxon being listed as "accepted" more than once under different generic combinations or different spellings. For example, both *Monodonta nigerrima* and *Diloma nigerrima* being listed as valid would be a duplicate, because both are based on *Turbo nigerrimus* Gmelin, 1791. *Tegula nigerrima* is not a duplicate of those names, however, as it is based on *Trochus nigerrimus* Gmelin, 1791, a different taxon. Similarly, unresolved homonyms would not be counted as duplicates: *Calliostoma canaliculatum* (Lightfoot, 1786) and *C. canaliculatum* (Sasao and Habe, 1973) are both accepted names in WoRMS because no replacement name is available for the junior secondary homonym.

Each epithet was used in truncated form in a "contains" search in the WoRMS "Scientific Name" field, "pulch", for example, matching *pulcher, pulchra, pulchrum, pulchellus, pulchella, pulchellum*, etc. The rank of the higher taxon in which the search was to be run (family, superfamily, order, etc.) was judged by the taxon. An epithet in Turridae, for example, would be searched in Conoidea, whereas one in Pyramidellidae would be searched in Gastropoda, as it might also be listed in Aclidae or even Rissoinidae.

Randomization was implemented by importing into Microsoft Excel 2010 all records from WoRMS with the target status (*e.g.*, verified, accepted species of bivalve) and assigning each record a random number using the "rand()" function. The records were then sorted lowest to highest by that number, and the first n records were evaluated, with $n = 50$ for verified accepted bivalves, $n = 100$ for unverified accepted gastropods and verified accepted bivalves and $n = 200$ for verified accepted gastropods.

Percentages of erroneous records were calculated as (duplicates/2 + synonyms + out of scope)/n. Duplicates were halved because a duplicate record is twice as likely as an unduplicated record to be selected at random. (In a database with 50% single records and 50% duplicate records, 25% of the records must be deleted to get rid of the duplicates.) The effect of triplicate records was assumed to be negligible, allowing calculation of standard errors based on the binomial distribution. For chi square tests, two-tailed p values were determined using the GraphPad online calculator, with degrees of freedom one less than the number of categories (http://graphpad.com/quickcalcs/chisquared1.cfm).

To find species missing from WoRMS, I sampled marine gastropod species at random from the ANSP mollusk collection inventory (see Introduction), as follows. For each species, the number of lots in the collection was determined, and a random number assigned. The spreadsheet was then sorted, first by number of lots, then by random number, and the first 200 names in each of five groups were selected starting at 1 lot, 5 lots, 10 lots, 20 lots and 40 lots, representing a gradient of "rare" to "common". The latter two groups spanned ranges of 20–22 and 40–51 lots, because ANSP did not have 200 marine gastropod species with the starting number of lots. The resulting 1000 names were matched against the WoRMS database using the genus and species epithets and the WoRMS automated matching tool. Names that matched exactly were accepted as matching without further investigation; names that matched by pattern or phonetics were checked to confirm matching. Unmatched names were queried manually in WoRMS using the truncated epithet, as described above.

Names for which no match could be identified in WoRMS were searched online in Google Books, Google Scholar, and the Zoological Record to determine their status as valid or synonym. If no treatment since 1950 was found, or if sources conflicted as to status, with one not clearly more reliable than another, I assigned a status of *nomen dubium* or *taxon inquirendum*. If no treatment whatsoever was found, I considered the name a manuscript (unpublished) name.

ANSP does not yet have a standardized bivalve inventory so to estimate completeness of bivalves in WoRMS I queried the main collection database to generate a list of lots by name. I sorted the list by epithet within each bivalve family to group variant spellings. From this list I chose 100 rare nominal species, deliberately avoiding well known groups such as the Cardiidae and Pectinidae and well-covered geographic areas. Their names were evaluated in the same way as names of gastropods.

The number of named terrestrial pulmonate species was estimated by totaling the stated minimum and maximum estimates of species diversity for each genus-group taxon treated by Schileyko (1998–2007). Results were summed by family and compared to tallies of species listed as valid in various published treatments. The number of named terrestrial

operculate species was estimated from the average completeness of the ANSP collection for terrestrial taxa, which in turn was estimated from the collection inventory by querying for the number of species in each family and comparing it to the published estimates of diversity.

I have used "recent" in the sense of "extant in the last 500 years", rather than "Holocene". Species known to have gone extinct during that period are included in the totals reported here, although their exclusion would not have a significant effect on the overall estimate of diversity, there being fewer than 600 historical extinctions of mollusks documented thus far (Régnier *et al.* 2009). In discussing higher taxa, I have used a mixture of clades (*e.g.*, Neogastropoda) and grades (*e.g.*, Ptenoglossa) for ease of reference.

RESULTS

Marine Mollusca

On 25 October 2013, WoRMS contained 43,696 accepted species of Mollusca, according to its summary statistics page. The totals stated for component taxa of Mollusca in the WoRMS taxon tree totaled 43,699 (Table 1). This slight discrepancy probably results from WoRMS doing summary calculations on a live database, in which entries might change or be added while the calculations are being done.

The WoRMS summary statistics are supposed to exclude species that are not recorded as marine or brackish (*i.e.*, terrestrial or freshwater) and those that are fossil only. Table 1 shows how many species are excluded in each category by taxon. The WoRMS summary figures by taxon (Table 1) compare well with those directly queried. The main difference is in counts of species that are freshwater and brackish, but not marine. WoRMS includes them; I have excluded them if they are part of predominantly freshwater radiations (*e.g.*, Hydrobiidae) and included them if they are part of mainly marine radiations (*e.g.*, Teredinidae). Relative to the counts in WoRMS (43,699), about 220 species are excluded, giving a total of 43,480.

Erroneous listings

Next, I estimated the prevalence of duplicate names and other errors that inflate the count of species (Table 2). I found by random sampling that among unverified names for gastropods ($n = 4,096$) about 5.5 ± 2.3 % were erroneous, compared to $24 \pm 6\%$ for bivalves ($n = 222$). Among verified names, about $2.5 \pm 1.1\%$ for gastropods and $2.0 \pm 1.4\%$ for bivalves were erroneous. Based on the standard errors, these percentages suggest that incorrect listings inflate the totals for gastropods by 937 ± 328 species and for bivalves by 210 ± 111 species. Duplicates were also found in the minor classes by taking a complete list of accepted species in each class and

sorting by specific epithet, but these were minor in effect (39 duplicates, 1.4% across minor classes). Altogether, erroneous listings inflate the numbers by about 3% and the total in WoRMS should be adjusted from 43,480 to $42,294 \pm 346$ species. See Appendix 1, Supplementary Documents (http://www.bioone.org/doi/suppl/10.4003/006.032.0204/suppl_file/Rosenberg_2014_suppl.xlsx) for a list of the names sampled.

Missing names

In the random samples from the ANSP collection of 200 names of gastropods in different abundance classes, the percentage of names missing from WoRMS increased with the rarity of the name, with more than 10% missing in the rarest group, but when only valid names (Table 3, Fig. 2) were considered, there was not a significant difference among the 5 classes (chi-square, 8.231; $p = 0.0835$). Regrouping the data into two classes ("rare", 1 and 5 lots and "common", 10, 20+ and 40+ lots), did give a significant chi square (6.981; $p = 0.0082$). The chi-square calculations took into account that N differed slightly among the frequencies classes (see Table 3). It was less than 200 in some cases because I excluded sampled names that proved to be outside of scope for WoRMS (manuscript, fossil, or terrestrial) or that were "double hits"— different names from the ANSP collection that were synonyms of the same name in WoRMS and thus not independent tests for missing names.

The selected sample of bivalve names (purposely biased toward rare names) returned values consistent with the rarest two size classes for gastropods (Fig. 2), but not the commoner size classes, in terms of the percentage of names missing from WoRMS. With all data pooled, $2.75 \pm 0.50\%$ (*SE*) of the sampled lots represent valid species that were missing from WoRMS, so $1,086 \pm 198$ should be added to the total estimated in Table 2. This gives an estimate of $43,380 \pm 398$ species for total marine diversity (Table 4). With data separated into rare and common species (Table 3), an overlapping estimate of $43,610 \pm 465$ results (Table 4). I have used the latter estimate in subsequent calculations herein. See Supplementary Documents (http://www.bioone.org/doi/suppl/10.4003/006.032.0204/suppl_file/Rosenberg_2014_suppl.xlsx) for a list of the gastropod (Appendix 2) and bivalve names (Appendix 3) sampled and their statuses.

Table 5 shows the gastropods species selected from the ANSP collection distributed by taxonomy, with the percentage of names missing from WoRMS. Figure 3 shows that only Opisthobranchia and Pulmonata are likely to exceed 5% missing from WoRMS. Table 6 shows that some taxa differ significantly from the even distribution expected between the abundance classes, indicating that selecting species randomly with respect to abundance (number of lots) does not result in random selection with respect to

Table 1. Species diversity by taxon from queries in the World Register of Marine Species (WoRMS). "WoRMS summary" = species tallies from WoRMS taxon tree; "species accepted" = accepted taxa of species rank with no other restriction; "unverified" = status as accepted not verified by taxonomic editor; "> 1 June 2012" = growth of database from addition of newly described species after that date; "quarantined" = names formally quarantined in WoRMS as problematic (*e.g.*, Stuardo manuscript names in Bivalvia) or of unknown status; "fossil only" = not recent; "not marine" = freshwater only, terrestrial only, or brackish and freshwater with clade primarily freshwater; "revised total" = species accepted minus quarantined, fossil only and not marine, adjusted for any overlaps among those categories.

Taxon	WoRMS summary	Species accepted	Unverified	> 1 June 2012	Quarantined	Fossil only	Not marine	Revised total
Gastropoda								
Patellogastropoda	328	335	69	0	0	7	0	328
Vetigastropoda	3,425	3,435	555	79	0	11	0	3,424
Cocculiniformia	49	49	0	0	0	0	0	49
Neomphalina	44	44	0	0	0	0	0	44
Neritomorpha	200	208	26	0	0	0	8	200
Caenogastropoda								
Abyssochrysoidea	34	34	0	0	0	0	0	34
Campaniloidea	15	15	0	0	0	0	0	15
Cerithioidea	593	675	123	4	0	0	84	591
Cyclophoridea	1	1	0	0	0	0	1	0
Epitonioidea	772	774	513	3	0	3	0	771
Eulimoidea	1,005	987	375	33	0	7	0	980
Triphoroidea	1,388	1,391	46	102	0	3	0	1,388
Viviparoidea	3	7	3	0	0	0	7	0
Littorinimorpha	5,083	5,447	801	22	0	27	389	5,031
Neogastropoda	12,578	12,598	907	262	0	113	2	12,483
Heterobranchia								
non-pulmonate	6,989	7006	611	42	2	12	15	6,977
Pulmonata	268	449	67	13	0	0	195	254
Subtotal, Gastropoda	32,775	33,455	4,096	560	2	183	701	32,569
Bivalvia	8,090	8,275	222	73	89	58	50	8,078
Cephalopoda	832	835	239	1	3	1	0	831
Polyplacophora	989	989	6	0	0	0	0	989
Aplacophora								
Caudofoveata	132	132	0	0	0	0	0	132
Solenogastres	273	273	0	4	0	0	0	273
Monoplacophora	31	31	0	1	0	0	0	31
Scaphopoda	577	579	0	2	1	1	0	577
Total	**43,699**	**44,569**	**4,563**	**641**	**95**	**243**	**751**	**43,480**

taxonomy. Neotaenioglossa is biased toward higher abundance and Ptenoglossa and Heterostropha are biased toward lower abundance.

Terrestrial diversity

Summation of stylommatophoran diversity from Schileyko (1998–2007) showed 17,575 to 18,613 species-group taxa. Schileyko's counts often combined species and subspecies, however, and so could be inflated. To test this, I compared his figures to those reported in various species catalogues published over the last 35 years (Table 7). Those catalogues treated 34 families and listed 11,116 species, 10% more than

the higher end of Schileyko's estimate, corresponding to about 20,500 species for all stylommatophorans.

The inventory of the ANSP collection recorded 10,021 stylommatophoran species, excluding Cerionidae (see Discussion) and slugs that lack external shells (since the inventory currently does not cover the alcohol preserved collection). In the families compared in Table 7, ANSP holds 5,844 species. It is therefore between 50.2% (10,021/19,979) and 61.5% complete (5,844/9,495) for described stylommatophoran species. The 2,323 terrestrial operculate species inventoried in the ANSP collection thus extrapolate to 3,777 to 4,627 species worldwide, if they have the same proportional completeness as the

Table 2. Counts of species recognized as valid in WoRMS, adjusted for duplicate and extralimital listings (*e.g.,* freshwater, terrestrial, and fossil only not flagged as such). "June 2012" and "Oct. 2013" = species counts as of those dates; "new spp." = species named after 2011 added after 1 June 2012; "growth w/o new" = increase (or decrease) in valid species between June 2012 and Oct. 2013 from addition of species previously missed, changes in synonymy and correction of errors; "unverified" and "verified" refer to whether a listed species was verified by a taxonomic editor; "wrong" = percentage of erroneous listings; "*SE*" = standard error of the percentage; "adjusted" = estimate of valid species using upper and lower bounds based on the standard errors of percentage wrong.

Taxon	June 2012	Oct. 2013	new spp.	growth w/o new	Unverified total	wrong	*SE*	Verified wrong	*SE*	Adjusted ± *SE*
Gastropoda	28,861	32,569	560	10.9%	4,096	5.5%	2.28%	2.5%	1.1%	31,632 ± 328
Bivalvia	7,825	8,078	73	2.3%	222	24%	6.04%	2.0%	1.40%	7,868 ± 111
Cephalopoda	821	831	1	1.1%	239	4.2%	–	1.2%	–	814 ± 0
Polyplacophora	972	989	0	1.7%	6	100%	–	1.1%	–	972 ± 0
Aplacophora	402	405	4	-0.2%	0	–	–	0.2%	–	404 ± 0
Monoplacophora	30	31	1	0.0%	0	–	–	0.0%	–	31 ± 0
Scaphopoda	577	577	2	-0.3%	0	–	–	0.7%	–	573 ± 0
Total	**39,488**	**43,480**	**641**	**8.5%**	**4,563**	**6.5%**		**2.3%**		**42,294 ± 346**

stylommatophorans at ANSP. This seems a reasonable assumption based on the Annulariidae, the only major operculate family for which a current revision is available. Watters (2006) recognized 669 to valid 692 species of annulariids; ANSP holds 399 of these (57.7% to 59.6%), following Watters synonymies.

DISCUSSION

Marine Mollusca

Analysis of the WoRMS database shows that the estimated number of species missing from WoRMS, about 1,200 species is essentially the same as the number of erroneous and duplicate listings that inflate the totals (as of October 2013): about 1300 species. The total found by querying WoRMS, 43,480 is the similar to the total after subtracting errors and adding missing species: 43,600 ± 465. Converting the standard error to a 95% confidence interval (multiplying by 1.96) gives an overall estimate of 43,600 ± 900 named marine mollusk species.

Duplicates were higher in bivalves because most verified names were imported at one time from Huber (2010, CD-ROM), and some preexisting unverified names in WoRMS were not checked against them. More surprising was that 2.5% of verified names in gastropods, and 2% in bivalves were erroneous, despite having been vetted by a taxonomic

Table 3. Percentages of names missing from WoRMS based on sampling the ANSP collection inventory by frequency classes for gastropods and for selected rare bivalves (see methods). "All pooled" combines data for gastropods and bivalves; "rare" combines data from gastropods with 1 and 5 lots with data from bivalves; "common" combines data from gastropods with 10 or more lots. N is less than 200 for some gastropod groups due to exclusion of unavailable (manuscript or nude), extralimital (non-marine or fossil only) and duplicate names.

Group	*N*	Missing names Total	%	*SE*	Missing valid names Total	%	*SE*
Gastropods, 1 lot	194	22	11.3%	2.3	7	3.6%	1.3
Gastropods, 5 lots	198	14	7.1%	1.8	10	5.1%	1.6
Gastropods, 10 lots	199	10	5.0%	1.5	4	2.0%	1.0
Gastropods, 20+ lots	199	5	2.5%	1.1	3	1.5%	0.9
Gastropods, 40+ lots	200	4	2.0%	1.0	2	1.0%	0.7
Rare bivalves	100	8	8.0%	2.7	4	4.0%	2.0
All pooled	1090	62	5.69%	0.70	30	2.75%	0.50
Rare (gastropods + bivalves)	492	44	8.90%	1.29	21	4.27%	0.91
Common (gastropods)	598	18	3.01%	0.70	9	1.51%	0.50
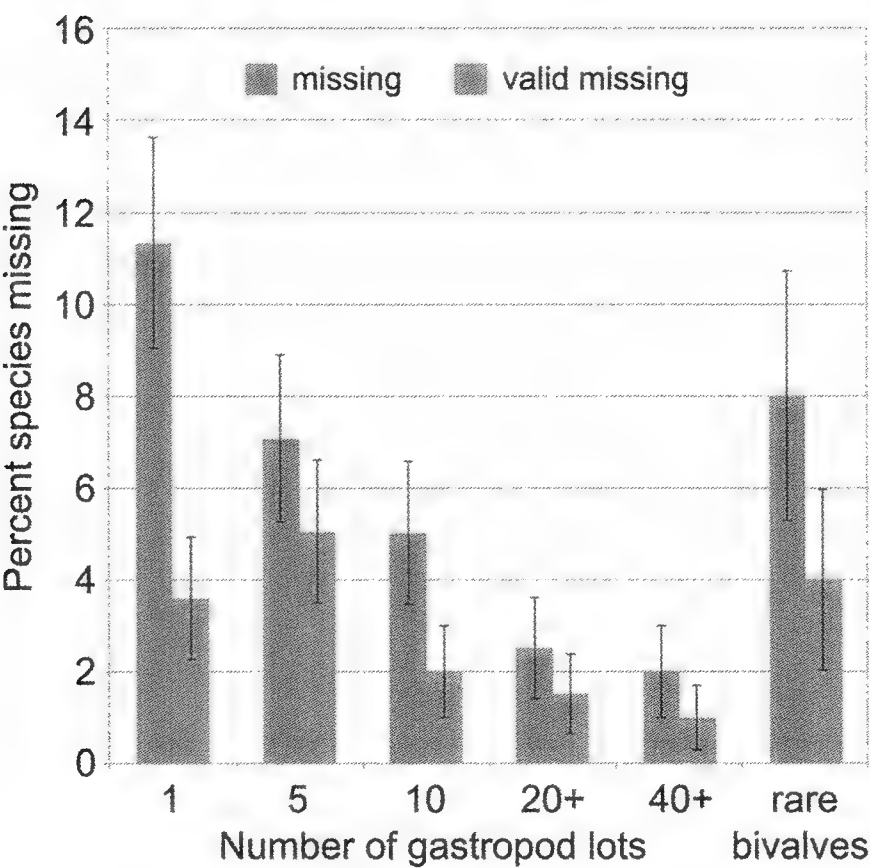
Figure 2. Percentage of names randomly sampled from ANSP collection by abundance classes (1 to 40+ lots) that are missing from WoRMS, comparing all missing names to valid missing names. Error bars show standard errors. Names missing among rare bivalves selected from the ANSP collection are shown from comparison. Data shown in Table 3. (Color shown in electronic version only).

editor. Across verified and unverified names the most common error was listing a name as valid in two different genera, but I found instances of names referring to taxa that were non-marine, fossil only, synonymous, dubious, and taxon inquirendum and in one case non-existent (these are detailed in the Appendix 1, Supplementary Documents, http://www.bioone.org/doi/suppl/10.4003/006.032.0204/suppl_file/Rosenberg_2014_suppl.xlsx).

The estimate of 43,600 marine species is substantially lower than that of Bouchet (2006): 52,525 species (which I agreed with at the time), based on summing estimates across "essentially non-overlapping" geographic regions. The total from his footnote 18 is actually 54,269 as North Pacific (1,744) was omitted. Including the North Pacific species along with the 3,800 marine species named from 2006 to October 2013 (queried in WoRMS), Bouchet's estimate corresponds to about 58,000 species, 33% higher than estimated

herein. Which estimate is correct determines which marine phylum is most diverse: arthropods are currently listed as having 56,000 species in WoRMS.

Bouchet's estimate has two main uncertainties: the size of the Indo-Pacific fauna and the degree of overlap with other regions, which was not accounted for. Bouchet considered that the Indo-Pacific molluscan database (IPDB) (Rosenberg et al. 2002) was about two-thirds complete, and thus extrapolated from its total of 24,269 to a figure of 32,000 species. To estimate completeness, I took a random sample of 200 of the 1,000 gastropods names from Table 3 and assigned them to ocean regions, finding 103 from the Indo-Pacific. (A second random sample was needed because I had not saved the random numbers of the original selection.) Of these, 18 were missing from IPDB, including 8 valid species, which corresponds to 7.8 ± 2.6 % missing, a lower percentage than any of the principals of IPDB had thought likely. This percentage missing yields an adjustment to 26,000 rather than 32,000 species.

The other issue is overlap between ocean regions. IPDB includes information on other regions that Bouchet tallied. It records 593 species from New Zealand, 1293 from South Africa (1137 tropical and 224 temperate, with some overlap), and 213 from the Eastern Pacific. (I compiled these using the "Browse Geographic Regions" feature of the website and removed duplicates and non-species level taxa manually.) IPDB also contains a large portion of the North Pacific fauna, by virtue of an import of data from Higo, Callomon and Goto (1999) for Japan. It cannot be readily queried to parse out the temperate species, as many were not given geographic flags, but if one-third of the north Pacific fauna is in northern Japan, then there is an overlap of about 600 species with IPDB. The Western Atlantic fauna has 21.7% overlap with the fauna of other regions (query of Malacolog 4.1.1, Rosenberg 2009), which corresponds to 1,339 out of 6,170 species listed by Bouchet. There is also overlap between the West African fauna and the European and South African faunas, which, if taken as 20%, represents 500 species. Summing up these various overlaps yields a total of 4,538. There are additional overlaps: North Pacific with European, and Antarctic and

Table 4. Total number of marine mollusks including estimate of species missing from WoRMS, based on adjusted values from right-most column in Table 2, with minor classes totaling 2,794. The first calculation uses the pooled value for percentage missing from Table 3; the second assumes rare taxa are more likely to be missing from WoRMS than common taxa and uses the corresponding percentages from Table 3. The total number of gastropods plus bivalves is parsed into rare vs. common in the ratio 66% to 34% observed in the ANSP for species with ≤ 7 lots and ≥ 8 lots. Standard errors were propagated as the square root of the sum of squares across columns and by addition within columns.

Group	WoRMS adjusted	Missing %	Missing	Total	Plus minor classes
Gastropoda + Bivalvia	39,500 ± 346	2.75 ± 0.50%	1086 ± 198	40,586 ± 398	43,380 ± 398
Common	13,430 ± 118	1.51 ± 0.50%	203 ± 67	13,633 ± 136	43,610 ± 465
Rare	26,070 ± 228	4.27 ± 0.91%	1113 ± 228	27,183 ± 329	

Table 5. Gastropod names from Table 3 regrouped to show percentage missing from WoRMS by taxonomy; N = number of species sampled. Opisthobranchia is used in a broad sense to include Acteonidae and Ringiculidae (2 species of each); Heterostropha contains the rest of the lower Heterobranchia (mainly Pyramidellidae and Architectonicidae); Pulmonata includes Ellobiidae, Siphonariidae and Amphibolidae. One heteropod and one stylommatophoran (miscoded as marine in the ANSP taxonomic dictionary) are not shown. The arrangements of patellogastropods and some ptenoglossans (Triphoridae) in the ANSP collection were updated against WoRMS in 2012 before work on this manuscript began, so the percentage missing from WoRMS may be artificially low for those taxa, however, the overall effect is minor, since only 1 or 2 names would be involved given the low N for those taxa.

Group	N	Missing names			Missing valid names		
		Total	%	SE	Total	%	SE
Patellogastropoda	26	0	0	0	0	0	0
Vetigastropoda	123	11	8.9%	2.6	5	4.1%	1.8
Neritogastropoda	6	2	33.3%	19.2	2	33.3%	19.2
Neotaenioglossa	207	9	4.3%	1.4	3	1.4%	0.8
Ptenoglossa	48	1	2.1%	2.1	1	2.1%	2.1
Neogastropoda	484	15	3.1%	0.8	8	1.7%	0.6
Heterostropha	41	1	2.4%	2.4	1	2.4%	2.4
Opisthobranchia (*s.l.*)	36	8	22.2%	6.9	3	8.3%	4.6
Pulmonata	15	5	33.3%	12.2	3	20.0%	10.3

Magellanic with New Zealand; also some species in IPDB have distributions including South Africa or New Zealand that are not flagged. I therefore use 5,000 species as an overall estimate of the degree of overlap across the oceanic regions.

Combining this overlap with 6,000 fewer Indo-Pacific species (32,000–26,000), gives a revised estimate of 47,000 marine mollusk species (58,000–11,000) based on the adjusted Bouchet data. This is still 3,400 higher (7.4%) than estimated herein. As with the WoRMS database, IPDB has some known duplication and also includes some non-marine taxa, but this seems to have been taken into account: the IPDB website lists 28,357 species "in current use" as of May 2006, about 4,000 more than the base Bouchet used. Therefore, either IPDB has additional, unrecognized duplication, the degree of overlap among faunas is greater than estimated above, the WoRMS database is missing more taxa than estimated above, or the situation is some combination of these possibilities.

All of the 26 species missing from WoRMS identified by randomly sampling the ANSP collection are from the Indo-Pacific, but only 52% (103/200) of the sample was from the Indo-Pacific. Of the non-valid missing names (synonyms and alternate combinations) 39% were not Indo-Pacific: Eastern Atlantic (16), Western Atlantic (12) and Eastern Pacific (23), versus Indo-Pacific (80). This reflects the prioritization of entry of valid names over synonyms by the WoRMS editors in order to reach an estimate of molluscan diversity. The random sampling done herein encompassed all ocean regions, but found gaps for valid names only in the Indo-Pacific. Overall, WoRMS is missing about 3% of marine mollusk species (1,300/43,600), with the main gaps in the Indo-Pacific. If

that fauna comprises 50% of marine mollusks, then about 6% of Indo-Pacific mollusk species are still missing from WoRMS. IPDB is currently being merged to WoRMS, which will fill in some missing species.

A major assumption in my analysis is that the ANSP collection and WoRMS do not have correlated weaknesses. Suppose that a particular family has 1,000 species, but ANSP and WoRMS each have only 200 names in that family. At random one would expect 40 names in common (20% of 200), but collections are surely biased toward common species, and a literature database might also have the same bias: common species are cited more often so there are more ways to encounter them to include them in the database. If such correlation led to there being 80 species in common within the target family instead of 40, the estimated percent missing from WoRMS would be 60% (120/200) instead of 80%.

Figure 2 shows that rare species (those in the lower abundance classes) are indeed more likely to be missing from WoRMS and Table 4 shows that when this is taken into account, the estimate of diversity is higher, but not by a significant amount. The rarest species, those represented by only 1 lot at ANSP, actually have a lower percentage missing from WoRMS (3.6%) than the next rarest class (5 lots, 5.1%), but the difference is not significant (Table 3, Fig. 2). The trend is interesting, however, because it suggests that there is not a separate class of ultra-rare species that would throw off estimates of diversity. Rather, there is a distinction between nominal species and valid species. Nominal species represented by only 1 lot in the ANSP collection are more likely to be spurious: dubious names, or synonyms not flagged as such. (Manuscript names are also spurious, but they are not

Figure 3. Percentage of names randomly sampled from ANSP collection missing from WoRMS by group (clade or convenient grade). Patellogastropoda not shown as no missing names were found; Neritogastropoda not shown as sample too small to be meaningful. Error bars are standard errors. Data shown in Table 5. (Color shown in electronic version only).

Random sampling of the ANSP inventory by abundance did not lead to random sampling by taxonomy (Table 6). Chi square tests showed significant departures from null distributions for three taxa, with Neotaenioglossa biased toward higher abundance classes and Heterostropha and Ptenoglossa (Epitonioidea, Eulimoidea, Triphoroidea) biased toward lower abundance classes. This may reflect a biological signal as many neotaenioglossans are herbivorous and therefore expected to have higher abundance because of lower trophic level, whereas ptenoglossans are generally parasitic, with lower abundance because of their higher trophic level. Despite this pattern, neither Ptenoglossa nor Heterostropha had a significantly higher proportion of species missing from WoRMS than expected (Figure 3). The greatest proportions of missing species were seen in Neritogastropoda and marine pulmonates, but these are both relatively low diversity groups, so their large error bars (Table 5) add little uncertainty to the estimate of diversity.

The best known group of gastropods in terms of species diversity is certainly the Neogastropoda, with recent treatments and catalogues by Tucker (2004, Turridae *sensu lato*), Snyder (2003, Fasciolariidae), Cossignani (2006, Marginellidae), Petit and Harasewych (2005, Cancellariidae); and Sterba (2004, Olividae), and unpublished lists by R. Houart on Muricidae, K. Monsecour on Columbellidae and K. Fraussen on Buccinidae all incorporated into WoRMS. I estimate that WoRMS is missing only 1.7 ± 0.6% (Table 5) of neogastropods. Neotaenioglossa, Ptenoglossa and Heterostropha are not significantly different from Neogastropoda in percentage of species missing (overlapping errors bars in Fig. 3), so WoRMS is generally very complete for high diversity groups of gastropods. The exception is Opisthobranchia with 8.3 ± 4.6 % missing. This percentage applies only to shelled opisthobranchs, however, as the ANSP inventory has not been extended to the alcohol collection as yet.

To assess the completeness of nudibranchs in WoRMS, I used listings in IPDB. IPDB doesn't have "Nudibranchia" in its taxonomic hierarchy, instead linking families directly to Opisthobranchia, so there was no quick way to do random sampling. Also, only in the Opisthobranchia, IPDB uses "Described" in the subgenus field to flag named species. I therefore searched for "Described" to get a list of Opisthobranch genera and took all species in nudibranch genera within those, alphabetically from A through G, getting a sample of 553 names. I eliminated nomenclatural duplicates from this list manually, leaving 528 names. Combining

available names and so are not nominal species; they were excluded from figures and calculations herein, as noted above.) Nominal species represented by five lots have generally come into the collection from several sources, so their names are more likely to represent valid species. Names that are seldom cited are less likely to represent valid species, and are more likely to be missing from WoRMS, which has prioritized the entry of valid names. Also, rare names are less likely to be correlated between databases and collections and so are less likely to mislead random sampling.

Table 6. Distribution by frequency class for gastropod names from Table 3. Chi square with 4 degrees of freedom, two-tailed t-test, significant p values marked with asterisk. Random selection by number of lots does not result in random selection by taxonomy, which would give an even distribution.

Group	1 lot	5 lots	10 lots	20+ lots	40+ lots	Chi squared	p value
Patellogastropoda	3	6	6	7	4	2.077	0.722
Vetigastropoda	22	23	26	30	22	1.919	0.751
Neotaenioglossa	39	32	32	43	61	13.749	**0.008***
Ptenoglossa	17	14	9	1	6	16.167	**0.003***
Neogastropoda	82	96	112	104	90	5.669	0.225
Heterostropha	14	13	6	3	5	12.049	**0.017***
Opisthobranchia (*s.l.*)	10	7	6	8	5	2.056	0.726
Pulmonata	5	5	2	1	2	4.667	0.323

automated matching through the WoRMS portal and searching names in McDonald (2009), I determined that 59 of these represented valid species that were missing from WoRMS. In 61 cases one of the 528 names mapped to the same valid name as another. I excluded these as duplicates, so the percent valid names missing from IPDB is 12.6% [59/(528–61)]. Assuming that 60% of the 2121 nudibranchs in WoRMS are Indo-Pacific, about 160 are missing (2121*0.126*0.6). Assuming that no more than 2% are missing from the rest of the world, 180 are missing overall. About 60 of these are accounted for in the calculations in Table 4, so the overall effect on the estimate of global marine molluscan diversity is small. The overall percentage of nudibranchs missing (8.5%; 180/2121) is quite similar to the figure for shelled opisthobranchs, 8.3% (Table 5).

Another group which the ANSP inventory does not yet cover is Bivalvia, so I estimated its completeness by selecting 100 rare species as described in Methods. They appear to be drawn from the same distribution as rare gastropods, with 8 names missing, 4 of them valid (Fig. 2), so I combined data from gastropods and bivalves in estimating overall diversity. In my presentation of preliminary data for this study at AMS in 2012, I noted that the gastropod to bivalve ratio seemed to increase with the number of faunal provinces included, with Keen (1971) showing 3.0: 1 for the tropical Eastern Pacific, Higo, Callomon and Goto (1999) 3.4: 1 for Japan, which spans tropical temperate and boreal, and Rosenberg (2009, Malacolog) 3.9: 1 for the Western Atlantic (Greenland to Antarctic). This suggested that gastropods have smaller geographic ranges on average than bivalves. (I have since found that Nicol (1969) estimated this ratio as nearly 3.2 to 1, across various regional faunas.) Since the ratio for gastropods to bivalves in WoRMS was then 3.7: 1, I predicted that it would increase as WoRMS became more complete. Currently the ratio is 4.02: 1 (data from Table 2), suggesting that WoRMS may be reaching an equilibrium level, with completeness of gastropods and bivalves now similar. The same kind of analysis applied to the percentage of Neogastropoda among Gastropoda also suggests that WoRMS is nearly complete. In June 2012, WoRMS had 41.6% Neogastropoda, which is higher than in other large-scale works: Abbott (1974) 36.9% (1657/4490); Keen (1971) 37.2% (900/2421); Higo, Callomon and Goto (1999) plus Okutani (2000, for shell-less); 38.5% (2015/5235); and Rosenberg (2009), 39.6% (2047/5167). In the current study the percentage has dropped to 38.3%, which is within the range of the other works, as coverage of non-neogastropods has strengthened.

The minor classes are essentially complete in WoRMS, with Aplacophora and Scaphopoda showing slight decreases when newly described species are discounted (Table 2, "growth w/o new"), reflecting synonymization of species formerly recognized as valid. The flux of species in and out of synonymy will be an ongoing source of uncertainty in estimates of molluscan diversity. There are surely species currently listed as valid in WoRMS for which a published synonymization has been overlooked. Also, some recently named species will be prove to be synonyms: the apparently acceleration of naming reflected by the upswing in the naming curve since about 1990 (Fig. 1) is at least partially an artifact of insufficient time having passed for taxonomic revisions to reveal synonyms. Of 4,674 marine species named in the last ten years (2004–2013), 94 (2%) have already been synonymized (query of WoRMS). Balancing this effect to an unknown degree will be the removal of earlier named taxa from synonymy. The errors bars on my estimate of marine diversity do not account for the flux of synonyms.

While it is not currently possible to adjust the overall estimate of marine molluscan diversity to take into account this flux (which would require knowing an average lag time between original description and relegation to synonym), there is reason to believe the figure of 43,600 is not an underestimate: many species listed in WoRMS as accepted are actually *nomina dubia*. These are taxa that have not been treated as valid in many years, and many have never been reevaluated since they were named. For example most of the more than 200 pyramidelloid taxa named by Saurin (1958, 1959, 1962) are listed as accepted

Table 7. Stylommatophoran species diversity by family summed across the low and high estimates for genus-group taxon stated by Schileyko (1998–2007). "Other" shows species diversity from the listed reference. At the bottom "Families compared" gives the sum for families with data in the "other" column; this was also calculated excluding slugs for comparison to the ANSP inventory. The number in brackets at bottom right is the extrapolation of the sum to all families.

Family	Low	High	Other	Reference	Family	Low	High	Other	Reference
Bulimulidae	1754	1874	1493	Richardson 1995	Milacidae	43	44		
Clausiliidae	1331	1387	1278	Nordsieck 2007	Humboldtianidae	40	41	42	Richardson 1982
Camaenidae	1040	1073	1073	Richardson 1985	Athoracophoridae	38	40		
Ariophantidae	992	1034			Discidae	38	44		
Urocoptidae	991	1014	931	Richardson 1991	Epiphragmophoridae	37	38	61	Richardson 1982
Bradybaenidae	827	873	898	Richardson 1983	Strophocheilidae	34	34		
Subulinidae	802	827			Helicodontidae	30	37	65	Richardson 1980
Achatinellidae	631	654			Haplotrematidae	29	32	21	Richardson 1989
Streptaxidae	623	657	1127	Richardson 1988	Daudebardiidae	28	29		
Charopidae	532	545			Dorcasiidae	27	27		
Hygromiidae	511	557	1147	Richardson 1980	Truncatellinidae	26	27		
Endodontidae	440	457			Strobilopsidae	24	26		
Helicidae	396	433	606	Richardson 1980	Thysanophoridae	22	29	90	Richardson 1989
Zonitidae	381	408			Gymnarionidae	21	21		
Euconulidae	364	395			Helicodiscidae	21	24		
Amastridae	321	326	331	Solem 1990	Cochlicellidae	21	25	21	Richardson 1980
Oleacinidae	311	327			Anadenidae	19	21		
Enidae	301	340			Cochlicopidae	18	23		
Achatinidae	269	277			Parmacellidae	17	17		
Polygyridae	260	285	239	Richardson 1986	Monadeniidae	17	19	13	Richardson 1982
Vertiginidae	240	263			Philomycidae	15	22		
Chronidae	236	249			Spelaeodiscidae	13	14		
Helicarionidae	206	227			Caryodidae	12	12		
Pleurodontidae	186	203	220	Richardson 1985	Clavatoridae	12	12		
Helminthoglyptidae	177	182	206	Richardson 1982	Corillidae	12	12	11	Richardson 1986
Ferussaciidae	177	190			Ryssotidae	12	14		
Rhytididae	172	172	199	Richardson 1989	Sculptariidae	10	10	16	Richardson 1986
Orculidae	150	170			Megalobulimidae	10	10		
Partulidae	137	139	142	Richardson 1990	Chlamydephoridae	10	12	12	Richardson 1989
Succineidae	135	141			Oopeltidae	10	12		
Trochomorphidae	124	138			Trigonochlamydidae	9	9		
Xanthonychidae	123	127	107	Richardson 1982	Sphincterochilidae	8	10	33	Richardson 1980
Pachnodidae	122	136			Ammonitellidae	7	8	9	Richardson 1989
Dyakiidae	109	111			Pyramidulidae	7	10		
Gastrocoptidae	106	116			Megaspiridae	6	6		
Pupillidae	100	105			Draparnaudiidae	6	6		
Chondrinidae	97	97			Aillyidae	5	6		
Punctidae	97	100			Testacellidae	5	7		
Acavidae	96	96			Cystopeltidae	4	4		
Glessulidae	90	90			Ostracolethidae	4	4		
Oreohelicidae	90	90	60	Richardson 1984	Cerionidae	4	5		
Vitrinidae	84	90			Staffordiidae	3	4		
Plectopylidae	82	82	109	Richardson 1986	Rhysotinidae	2	2		
Hypselostomatidae	72	79			Boettgerillidae	2	2		
Sagdidae	68	84	114	Richardson 1986	Elonidae	2	2		
Valloniidae	63	64			Unknown	2	3		
Systrophiidae	60	65	146	Richardson 1989	Macrocyclidae	1	1		
Gastrodontidae	57	60			Micractaeonidae	1	1		
Ciliellidae	56	64	84	Richardson 1980	Thyrophorellidae	1	1		

Table 7. (Continued)

Family	Low	High	Other	Reference	Family	Low	High	Other	Reference
Arionidae	54	66			Papillodermidae	1	1		
Agriolimacidae	47	50	123	Wiktor 2000	Bielziidae	1	1		
Orthalicidae	47	102	89	Richardson 1993	**Total**	17,575	18,613		[all-families estimate]
Limacidae	46	48			Families compared	9542	10,112	11,116	[20,467]
Spiraxidae	45	53			Same, without slugs	9495	10,062	10,993	

in WoRMS, but most have never been cited subsequently. Similarly, many names of pyramidellids are propagated on faunal lists (*e.g.*, Keen 1971, Abbott 1974), but have not been revised since they were named. WoRMS currently lists more than 1,000 accepted species in *Turbonilla* alone, thereby overtaking *Conus* as the most diverse molluscan genus (Kohn 1992), but unlike the situation in *Conus*, most of the species are poorly known. In contrast to the situation in Pyramidellidae, when names of Turridae from Tucker (2004) were entered into WoRMS, nominal taxa that had not been allocated to a modern genus were omitted. This uneven treatment of seldom-cited and poorly understood taxa adds uncertainty to estimates of molluscan diversity.

Non-marine Mollusca

Lack of an overall database for non-marine mollusks prevents a statistical treatment such as that I applied to the marine mollusks. For freshwater diversity I have accepted the two most recent rigorous tallies: the estimate of 3,795–3,972 freshwater gastropods from Strong et al. (2008) and the enumeration of 1209 freshwater bivalves by Graf (2013). This gives an overall total for freshwater mollusks of about 5,100 species (Table 8), which is substantially lower than the 7,000 estimated by Lydeard et al. (2004). I am, however, in substantial agreement with the latter's estimate of 24,000 species for terrestrial mollusks, having reached a total of 24,500 ± 2,000. (The error figure here is merely a range of likely values rather than a statistical confidence interval.)

Summing diversity from Schileyko's work gives a total of 17,575 to 18,613 stylommatophoran species (Table 7). The tallies from the species catalogues total 11,116, about 10%, more than the high-end total from Schileyko for the treated families. This extrapolates to 20,500 for all stylommatophorans. A bracket of 19,000 ± 1,500 species spans from the low-end total from Schileyko to 20,500. Even this bracket might be low: most of the nomenclators considered are by Richardson, who treated all subspecies as synonyms, whereas the trend in land snail systematics is to elevate subspecies to full species. In compiling information from Richardson, I recorded tallies genus by genus, mapping the genera to the families that Schileyko placed them in to control for different classifications.

I excluded Richardson (1992) on Cerionidae, which, unlike his other catalogues, treated nominal taxa rather than valid taxa. Another factor is that Schileyko's totals are now an average of ten years old and Richardson's are 20–34 years old and many new species have since been named. To compensate for these effects, I shift the bracket upward by 1,000 species, to 20,000 ± 1,500 stylommatophoran species.

My estimate of terrestrial operculate diversity has two uncertainties. It is based on the percentage completeness of the ANSP collection for pulmonate species, which may not be a good estimator for operculate species. My field experience in Jamaica suggests that operculates have smaller geographic ranges on average than pulmonates, so they may tend to be rarer and therefore less well represented in collections. However, the percentage completeness of the operculate family Annulariidae in the ANSP collection, 57.7% to 59.6% is within the range of completeness of pulmonate groups (50.2–61.4%). The other uncertainty is in the assignment of some assimineids as terrestrial, freshwater or marine, however, the estimate of 4,200 ± 450 terrestrial operculate species should be broad enough to encompass this. The overall estimate of terrestrial diversity including non-stylommatophoran pulmonates, of which there are probably fewer than 200 valid species, is 24,500 ± 2,000 (Table 8).

Table 8. Recent Molluscan species diversity summed across estimates for marine, freshwater and terrestrial. Basis of estimates is discussed in the text, except for "Other terrestrial pulmonates". In Ellobiidae I estimated 40 Carychiinae (Weigand *et al.* 2011) and 10 Pythiinae that are not also marine and 130 Systellommatophora (Veronicellidae, Rathousiidae and Onchidiidae) (D. G. Robinson pers. comm.; Dayrat 2009). Total is rounded.

Group	Estimate
Marine mollusks	43,600 ± 900
Stylommatophora	20,000 ± 1500
Terrestrial operculates	4200 ± 450
Other terrestrial pulmonates	180 ± 50
Freshwater gastropods	3900 ± 100
Freshwater bivalves	1200 ± 50
Total	**73,000 ± 3000**

Overall Recent molluscan diversity

The overall total I estimate for described recent Mollusca, 70,000 to 76,000 species does not closely agree with any prior study and is lower than all recent estimates except that of Boss (1971). My previous estimate of 72,000 species (Rosenberg 1992) although within this range, does not match because many species have been named since then. If the naming patterns for freshwater and terrestrial taxa resemble those of marine taxa (Fig. 1), 72,000 species in 1992 extrapolates to 89,000 in 2013 (24% increase). Boss (1971, table 13) estimated 33,754 described species and 46,810 including undescribed species. He emphasized that many taxa have proven to have high synonym ratios when monographed, so for groups that had not been monographed recently he was conservative in estimating how many species might prove valid. However, many names in synonymy were not described as species but as varieties or forms, so the original hypothesis of the naming author was not that a species existed. In the Western Atlantic fauna, more than 60% of names introduced at the species rank are currently considered valid (query of Malacolog), so synonymy ratios must be used in the appropriate context.

If unrecognized synonyms and *nomina dubia* prove to constitute a substantial proportion of names currently treated as valid in molluscan species lists, mollusks may eventually lag behind chordates in terms of the number of valid species recognized. Currently, about 69,000 chordate species are recognized (Table 9), which is close to the lower end of my estimate for molluscan species. Also, naming rates for chordates are currently higher than for mollusks, about 750 species per year (Table 9), versus about 600 per year (467/year, 2004–2013, query of WoRMS, plus about 100 non-marine per year, query of Zoological Record), although the mollusks are generally regarded as having a higher level of undescribed diversity, as reflected in the many statements placing of total molluscan diversity at 200,000 species. The accelerating rate of chordate discovery is abetted by the accessibility of

comprehensive databases of available names, which allows more rapid assessment of whether an unidentified taxon has been already named, and by molecular techniques, which are not yet as pervasive in malacology.

Lydeard et al. (2004) noted that mollusks have the highest documented extinction rate of any group of organisms, comprising 42% of the 693 extinctions documented on the IUCN Red List in 2002, versus 33% for tetrapods. While this is still true in relation to tetrapods, at the phylum level, chordate extinctions exceed molluscan extinctions on Red List 2013.2, with 325 (41 %) vs. 310 (39%). This is because of the inclusion of fish; tetrapods remain at 33%. (No tunicates or cephalochordates are listed by the IUCN.) Yet many molluscan extinctions are overlooked: as shown by Régnier et al. 2009, almost half of molluscan extinctions are not listed by the IUCN. Only 6,809 mollusk species have been assessed for the Red List and of these 27% (1,812) are listed as data deficient whereas 37,280 vertebrate species have been assessed, with 16% (5,908) data deficient. Thus 45% of vertebrates but only 7% of mollusks have a conservation status in IUCN.

Since 99% of molluscan extinctions are of non-marine taxa (Lydeard et al. 2004), development of master lists of described terrestrial and freshwater species is a high priority for molluscan conservation. The framework is now in place for "MolluscaBase": in February 2014 the data management staff at Flanders Marine Institute, which hosts WoRMS, reached an agreement with the Mollusca editors to broaden coverage of WoRMS to all Mollusca, recent and fossil. Such biotic databases are crucial for more rapid progress in molluscan systematics and conservation (Rosenberg 1997). Although documented molluscan species-level diversity is not as high as previously thought, undescribed diversity may exceed known diversity, as suggested by the species discovery curve in Figure 1, ongoing expeditionary work (Bouchet 2006) and molecular studies showing much overlooked diversity and regional endemism (*e.g.*, Meyer, Geller and Paulay 2005). In providing authoritative summaries of taxonomic, geographic

Table 9. Chordate species diversity and naming rates. Naming rate is based on a ten year average for tunicates, fish, amphibians and birds, and from Uetz (2010) for reptiles. Total for mammals was increased by 180 to account for 6 years of naming since tally.

Taxon	Species	Species/year	Source
Tunicata	3026	20	WoRMS
Cephalochordata	30	0	WoRMS
Fish	33,065	417	Catalogue of Fishes
Amphibia	7,215	180	http://amphibiaweb.org/amphibian/newspecies.html
Reptilia	9,909	100	http://www.reptile-database.org/db-info/SpeciesStat.html
Aves	10,518	8	IOUC http://www.worldbirdnames.org/
Mammalia	5,600	30	Reeder, Helgen and Wilson (2007)
Total	69,363	755	

and biotic information, online databases have an essential role to play in accelerating discovery and description of molluscan diversity.

ACKNOWLEDGMENTS

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Remembering Roland

By James B. Wood[1] and Jennifer Mather[2]

[1] Waikiki Aquarium, University of Hawai'i, Honolulu, Hawaii 96815, U.S.A.

[2] University of Lethbridge, Lethbridge, AB T1K 3M4, Canada

Friend, colleague and fellow malacologist and teuthologist Roland C. Anderson of Seattle died suddenly in his sleep in February 2014 at his Whidbey Island cottage. He was 67.

The son of a sea captain, Roland grew up near the coast and was fascinated with marine life, especially mollusks, from an early age. He was an avid scuba diver and shell collector and wrote about local dive sites. Roland earned his undergraduate degree from University of Washington and went to work at the Seattle Aquarium. Later he earned his Ph.D. in Marine Biology from Greenwich University. He served on the executive boards, including a term as president, for both the Western Society of Malacologists and the American Malacological Society. Roland recently delivered the keynote speech on color change in cephalopods and passing cloud displays at the World Congress of Malacology in the Azores in July of 2013. He was the author of over 200 scientific articles, primarily on the behavior, husbandry and natural history of marine mollusks. His recent work includes papers on tool use and play in octopuses, their recognition of individual humans, sand digging in sepiolids and enrichment for captive cephalopods. The authors of this remembrance co-authored the popular book, "Octopus: The Ocean's Intelligent Invertebrate" with Roland

Roland was a featured scientist on the Nature special, "Tentacles," and was often quoted in the media. He worked at the Seattle Aquarium for over three decades, retiring in 2009. Many knew him as the senior octopus biologist at the Seattle Aquarium. Roland created and organized the Puget Sound octopus surveys, the Octopus Week events including the Octopus Blind Date on Valentine's Day, and the Giant Pacific Octopus conference. Throughout his career, Dr. Anderson was always curious, helpful to colleagues and full of new ideas both theoretical and applied. Roland's friends span the globe.

Roland was a good friend to many and fun to work with. He had a well-developed sense of humor. During our field work in Bonaire, Roland would bring a gelatinous "wall walker," chill it in the freezer and then slip it into someone's dive bootie (the approved technique was not to react but to quietly slip it into someone else's bootie). In his obituary, Roland is described as "a researcher, author, diver, connoisseur of desserts, curmudgeon......and friend."

He will be missed.
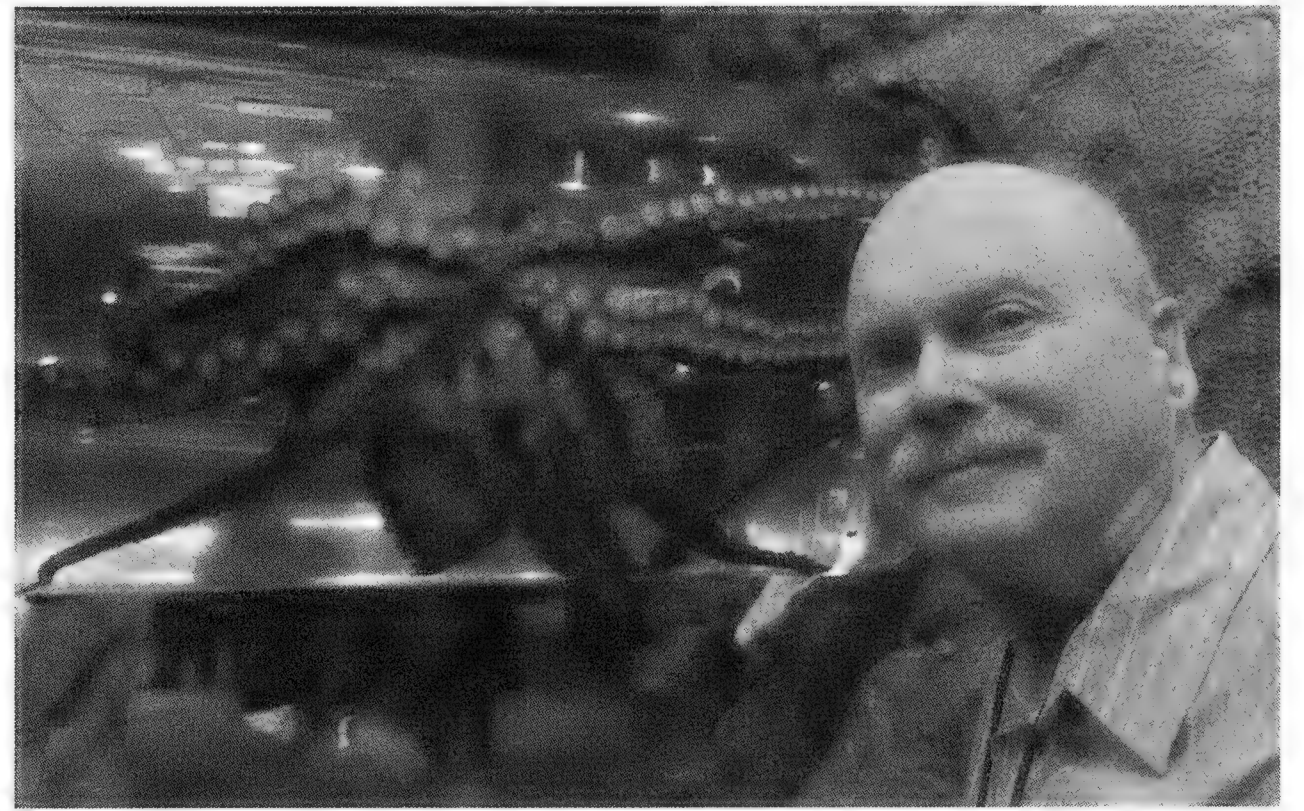
Roland Anderson at the Seattle Aquarium (Ken Lambert/The Seattle Times).

In Memoriam

Abbe, George Robert (1943–2013; U.S.A.)

Anderson, Roland C. (1946–2014; U.S.A.) (*Past President, AMS*)

Bern, Howard A. (1920–2012; Canada/U.S.A.)

Bosch, Donald Taeke (1917–2012; China/U.S.A./Oman)

Boss, Kenneth Jay (1935–2014; U.S.A.)

Burch, Beatrice LaRue (1917–2013; U.S.A.)

Clarke, Malcolm (1930–2013; United Kingdom)

Coleman, Neville (1938–2012; Australia)

Cosman, Dieter (1917–2012; Switzerland/U.S.A.)

Hoenselaar, Hendrikus J. (1926–2013; The Netherlands)

Huxley, Andrew Fielding [Sir] (1917–2012; United Kingdom)

Kilburn, Richard Neil (1942–2013; South Africa)

Kruglov, Nikolay D. (19**–2013; Russia)

Leobrera, Carlos Baldon ["Charlie"] (1935–2102; Philippines)

Nicolaci, Domenick D. (1920–2013; U.S.A.)

Pérez d'Angelo, Ernesto (1932–2013; Chile)

Petit, Richard Eugene (1931–2013; U.S.A.) (*Past President, AMS*)

Pizzini, Mauro (19**–2013; Italy)

Rapp, William F., Jr. (1918–2013; U.S.A.)

Ray, Sammy Mehedy (1919–2013; U.S.A.)

Rosenfield, Aaron (1924–2013; U.S.A.)

Rowan, William B. (1932–2013; U.S.A.)

Adolf Seilacher (1925–2014; Germany)

Stepczak, Kazimierz (1936–2013; Poland)

Szarowska, Magdalena (1952–2013; Poland)

Tippett, Donn Lloyd (1924–2014; U.S.A.)

Turgeon, Kenneth W. (1943–2013; U.S.A.)

Voisin, Michael (1954–2013; U.S.A.)

Partial list of colleagues who have died since 2012 compiled by Gene Coan and Alan Kabat, based on Coan and Kabat (2014) and subsequent reports.

Coan, E. V. and A. R. Kabat, 2014. 2,400 years of malacology, 11th ed., January 14, 2014, 1,128 pp. + 94 pp. [Annex 1 of Collations] + 65 pp. [Annex 2 – Küster Collation]. American Malacological Society: http://www.malacological.org/2004_malacology.html

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Guest Editors and Reviewers for 2011–2013

Guest Editors: Peter Marko and Alan Kohn (2011 AMB Symposium)

Reviewers (names in **bold** represent multiple manuscripts reviewed in 2011–2013)

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Berg, David
Bourdeau, Paul
Brown, Kenneth M.
Campbell, David
Cameron, Robert
Cerrano, Carlo
Chiba, Tomoki
Clemens-Seely, Katie
Davis-Berg, Elizabeth
Delgado, Marlon
Donovan, Deborah
Duda, Michael
Eernisse, Doug
Escobar, Juan S.
Fehrer, Zoltan
Frank, Hartmut
Frey, Melissa
Gillis, Patricia
Golladay, Steve
Gomes, Suzete
Graf, David
Guerra, Angel
Harding, Juliana M.

Hayes, David M.
Holland, Brenden
Howells, Bob
Jensen, Kathe
Johnson, Paul
Jones, Nick. J. E.
Kappes, Heike
Kasugai, Takashi
Kelley, Patricia
Kornilios, Panagiotis
Krug, Patrick
Lang, Brian
Lord, Josh
Lydeard, Charles
Mather, Jennifer
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McCarthy, Tom
Meyer, Marty
Miller, Luke
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Örstan, Aydin

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Vecchione, Michael
Vermeij, Geerat J.
Voltzow, Janice
Waldusser, George
Weaver, Kathleen
Willan, Richard

We apologize if we accidentally left any reviewer out of this list. Thank you all for your hard work!

American Malacological Society
81ST ANNUAL MEETING

University of Michigan Biological Station
Pellston, MI. August 28-31, 2015

The 81st meeting of the American Malacological Society (AMS) will take place from August 28th to 31st, 2015 at the University of Michigan Biological Station (UMBS) in Pellston, Michigan. The program will include symposia, contributed talks and posters, possible workshops, an auction to earn funds to support student research in malacology, social events and opportunities for local field trips. Information on meeting registration, events, schedules, accommodations and submission of titles will be presented on the AMS website (http://www.malacological.org/) in early 2015. Please contact Tom Duda (tfduda@umich.edu) for further information or if you are interested in organizing a workshop or symposium to be held at the meeting.

The UMBS, the meeting's venue, is nestled "up north" adjacent to Douglas Lake, one of the many inland lakes of Michigan, on approximately 10,000 acres of mostly secondary-growth forest near the northern edge of Michigan's Lower Peninsula (about 20 minutes south of the Mackinac Bridge). With about 150 buildings on site, including an auditorium and a large seminar room, classrooms, laboratories, cabins, dorm rooms, a dorm lounge and a dining hall, the station has served students and researchers for over 100 years (see http://www.lsa.umich.edu/umbs for more information on the UMBS). The self-contained and relatively rustic setting of the meeting should create an informal and lively camp-like atmosphere that will provide plenty of opportunities for interactions and conversation and impromptu field excursions. We look forward to seeing you "up north" next year!

INFORMATION FOR CONTRIBUTORS

Scope. The *American Malacological Bulletin* is the scientific publication of the American Malacological Society and serves as an outlet for reporting notable contributions in malacological research. Manuscripts concerning any aspect of original, unpublished research, important research notes, and detailed reviews dealing with molluscs will be considered for publication.

Format. Manuscripts and illustrations should be submitted electronically (in a MS Word document or PDF file with embedded figures). Text must be typed in 12 pt font on 8.5×11 inch (letter-sized) paper, double-spaced, with all pages numbered consecutively. Leave ample margins on all sides, and left-justify the text. Final submission of accepted, revised manuscripts must include the text, tables, etc. as a mandatory MS Word file on a CD, DVD, or e-mail attachment, along with high resolution TIFF files of all figures. Authors should make sure all figures have at least 350 dpi resolution. Please follow current instructions for authors given at the AMS website or at the back of recent issues of the Bulletin.

Sections. Text, when appropriate, should be arranged in sections as follows:

1. Cover page with title, author(s), address(es), e-mail address (of corresponding author), and suggested running title of no more than 50 characters including spaces. Authors should also supply 5 key words or short phrases, placed at the base of this page, for indexing purposes. Key words or phrases should not duplicate terms already in title.
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3. Text of manuscript starting with a brief introduction followed by materials and methods, results, and discussion.
   - Separate main sections of text with centered titles in bold face, capital letters.
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MATERIALS AND METHODS

Taxonomy
Animals
Cultured animals
Wild animals
Behavioral observations

RESULTS

---

4. Acknowledgments
5. Literature cited
6. Figure legends (together)
7. Tables (each on a separate sheet, headed by a brief legend)

Research Notes. Research Notes report results of complete studies but are less comprehensive than full-length articles. Research Notes are limited to 3000–4000 words including tables, figures, acknowledgments and literature cited. Separate headings are only required for the abstract, acknowledgments and literature cited. Authors may indicate the manuscript is to be considered as a Research Note. Editor-in-chief may also designate manuscripts as Research Notes upon review. All manuscripts are peer-reviewed.

Taxonomic Authorities. All molluscan binomens must include the author and date attributed to that taxon the first time the name appears in the manuscript, such as *Crassostrea virginica* (Gmelin, 1791). A comma is required between the authority and date. The full generic name along with specific epithet should be spelled out the first time that taxon is referred to in each paragraph. The generic name can be abbreviated in the remainder of the paragraph as follows: *C. virginica*. The taxonomic authorities of generic names must be provided if species names are not included. Please refer to recent issues for examples.

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Citation Format.

Beattie, J. H., K. K. Chew, and W. K. Hershberger. 1980. Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during summer mortality. *Proceedings of the National Shellfisheries Association* **70**: 184–189.

Hillis, D. M. 1989. Genetic consequences of partial self fertilization on populations of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* **7**: 7–12.

Seed, R. 1980. Shell growth and form in the Bivalvia. *In:* D. C. Rhoads and R. A. Lutz, eds., *Skeletal Growth of Aquatic Organisms*. Plenum Press, New York, New York. Pp. 23–67.

Yonge, C. M. and T. E. Thompson. 1976. *Living Marine Molluscs*. William Collins Son and Co., Ltd., London.

For more detailed examples of journal series, supplements, graduate theses, governmental reports, Internet citations, non-English citations, or other categories of references, please check the following AMS web page http://malacological.org/pdfs/amb/AMB_ref_examples.pdf.

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